Chiral resolution in supercritical carbon dioxide based on diastereomeric salt formation

PHD thesis

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TABLE OF CONTENTS
Introduction

1. Literature review
   1.1. Chirality
      1.1.1. Nomenclature of chiral compounds
      1.1.2. Quantification of chirality
   1.2. Chiral resolution
   1.3. Supercritical fluids
      1.3.1. Applications of supercritical carbon dioxide
      1.3.2. Antisolvent resolution
   1.4. Investigated racemates
      1.4.1. Ibuprofen
      1.4.2. cis-Permethyl acid
   1.5. Resolving agents
      1.5.1. 1-Phenylethanamine
      1.5.2. (S)-(+-)-2-(N-Benzylamino)butan-1-ol

2. Materials and methods
   2.1. Materials used
   2.2. View cell measurement methods
      2.2.1. Equipment
      2.2.2. Solubility measurement method
      2.2.3. Antisolvent screening method
   2.3. Batch resolution methods
      2.3.1. Equipment
      2.3.2. In vacuo method
      2.3.3. In situ method
      2.3.4. Gas antisolvent (GAS) method
   2.4. Supercritical antisolvent (SAS) method
      2.4.1. Results of apparatus development
      2.4.2. Measurement technique
   2.5. Analytical methods
      2.5.1. Chiral gas chromatography (GC)
      2.5.2. Powder X-ray diffraction (XRD)
      2.5.3. Scanning electron microscopy (SEM)
   2.6. Calculations
      2.6.1. Optical purity
      2.6.2. Yield
      2.6.3. Resolution efficiency

3. Results and discussion
   3.1. Resolution of ibuprofen with 1-phenylethanamine
      3.1.1. In situ method
      3.1.2. GAS method
      3.1.3. SAS method
   3.2. Resolution of cis-permethyl acid with (S)-(+-)-2-(N-benzylamino)butan-1-ol
      3.2.1. In situ method
   3.3. Resolution of cis-permethyl acid with (R)-(+-)-1-phenylethanamine
      3.3.1. GAS method
3.3.2. SAS method .......................................................... 84
3.4. Discussion .............................................................. 87
  3.4.1. Effect of racemate–resolving agent interaction .................. 87
  3.4.2. Effect of time ...................................................... 89
  3.4.3. Effect of pressure and temperature .............................. 90
  3.4.4. Effect of solvent composition ................................... 91
  3.4.5. Method development for antisolvent processes ................. 92

Conclusion 95

References 98

Article offprints 113
  BÁNSÁGHI et al.: J. Supercrit. Fluids, 2012 .......................... 114

Appendix A-1
  A. List of symbols ..................................................... A-1
  B. Original images ................................................... A-3
  C. Supplementary data ............................................... A-4
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INTRODUCTION

The production of optically active compounds is a key issue in contemporary chemical engineering [1]. Large numbers of optically pure ingredients are used in various sectors of the chemical industry, such as pharmaceuticals, foodstuffs, pesticides or cosmetics. The potentially harmful side effects of optical impurities require highly stereoselective approaches, while the large volume of production necessitates that syntheses be both economically and environmentally favourable.

Supercritical fluids possess several unique properties that make them attractive for applications in green chemistry [2]. Their properties are tunable by changing their pressure or temperature, allowing high degrees of optimization. Their low viscosity and lack of surface tension leads to improved diffusion rates, making them highly suitable for diffusion-limited processes such as extraction or heterogeneous catalysis. Expanding a supercritical fluid to below the critical pressure causes a rapid conversion to the gaseous phase, resulting in the precipitation of dissolved compounds with low residual solvent content, simplifying downstream processing.

Supercritical carbon dioxide is widely used in both laboratory and industrial scale processes [3–5]. In addition to the benefits mentioned above, it has a number of other properties that make it well suited to green chemistry applications. It is a non-flammable and non-explosive solvent that has no toxicity should residues contaminate products in trace amounts. It is abundantly available, easy to obtain and, if harnessed from biological processes – such as yeast fermentation – it does not increase atmospheric carbon dioxide levels. Its comparatively low critical temperature (31.0 °C) enables its application in the processing of heat-sensitive materials.

The objective of this thesis was identifying and developing chiral resolutions, based on diastereomer salt formation using supercritical carbon dioxide as a solvent or antisolvent, that are more environmentally friendly than existing methods, while yielding comparable results to said methods.

The resolutions of two model racemates have been investigated: ibuprofen, an over-the-counter analgesic and antipyretic drug and cis-permethric acid, a key intermediate in the synthesis of pesticides. The resolution processes involve the reaction of the racemate with an optically pure compound, the so-called resolving agent, in half-equivalent molar ratio according to a modified version of the Pope–Peachy method. The resolving agents used in the experiments were (R)-(+) -1-phenylethanamine and (S)-(−)-1-phenylethanamine, as well as (S)-(+) -2-(N-benzylamino)butan-1-ol. As a
result of the reaction between the racemate and the resolving agent, polar diastereomeric salts are formed, which can be separated from the relatively apolar unreacted racemate by extraction with highly apolar supercritical carbon dioxide.

In the \textit{in vacuo} method, the racemate and resolving agent are dissolved in an organic solvent separately, the solutions are mixed and the solvent is evaporated under vacuum. The resulting solids are placed into a high-pressure reactor and pressurized with supercritical carbon dioxide, allowing the composition of products to shift. The reactor is then washed with supercritical carbon dioxide to remove the unreacted enantiomers, leaving the diastereomers behind.

The \textit{in situ} method is a novel technique that utilizes supercritical carbon dioxide as a reaction medium for a heterogeneous reaction. The racemate and resolving agent are placed directly into the high-pressure reactor with no solvent and pressurized with supercritical carbon dioxide. Both components dissolve into the supercritical phase, react, and the formed diastereomers precipitate. The unreacted enantiomers left dissolved in carbon dioxide are removed by washing the reactor with supercritical carbon dioxide.

The resolutions have also been performed using antisolvent processes. Although widely utilized for various applications such as micronization, only a handful of attempts \cite{6–8} have been made to apply supercritical antisolvent processes to the separation of optically active compounds. These processes involve preparing a concentrated solution of the racemate and the resolving agent and contacting it with supercritical carbon dioxide, resulting in a solvent mixture of decreased solvent power from which the diastereomers precipitate. The unreacted enantiomers are separated by extraction with supercritical carbon dioxide. In the gas antisolvent process, the solution is placed into a high-pressure reactor and pressurized with supercritical carbon dioxide. In the supercritical antisolvent technique, the solvent is injected into a vessel that is maintained under pressure by a flow of supercritical carbon dioxide.

Experiments have been evaluated by determining the yields and optical purities of the products, as well as overall resolution efficiencies in certain cases. The diastereomeric salts have been analyzed with powder X-ray diffraction and scanning electron microscopy. The effects of operating conditions have been studied for all experimental techniques.
1. **LITERATURE REVIEW**

1.1. **CHIRALITY**

Asymmetric molecules which cannot be superimposed onto their mirror images are termed "chiral" molecules. The word originates from the Greek *kheir*, "hand", an intuitive example of mirror images that cannot be rotated to coincide with one another.

Chiral molecules belong in the larger group of molecules called stereoisomers. These are compounds that are identical in their chemical structure, but differ in the spatial arrangement of their constituent atoms. Typically, molecules have a number of stereoisomeric forms termed conformers, many of which may be non-superimposable onto their mirror images. However, the energy barrier between conformers is low enough to be regularly overcome, thus conformers are rapidly converted into each other, causing the time-averaged structure of the molecule to become symmetrical. Chiral molecules, on the other hand, are unique among stereoisomers in that they retain their asymmetry over time. Therefore, chiral molecules may exist in a number of distinct time-averaged structures, called configurations.

Compounds can possess a number of different types of chirality. Within organic chemistry, the most common type is point chirality, which arises from a single asymmetric atom, the so-called chiral center or stereogenic center. Typically, this is a carbon atom to which four different substituents are attached, although other atoms (e.g. phosphorous) can serve as chiral center as well. An asymmetric carbon atom, having four substituents, can exist in two configurations, an example of this is shown in Figure 1.

![Figure 1: 1-Chloroethanol, an example of point chirality. The molecule has one asymmetric carbon atom (marked with an asterisk), resulting in two optical isomers. As the two are mirror images, they constitute a pair of enantiomers.](image-url)

Other types of chirality include axial (such as the spiral arrangement of helicene molecules), planar (seen, for example, in metallocenes with asymmetrical aromatic ligands), or inherent chirality (e.g. asymmetric fullerenes). A special case of axial
chirality, termed atropisomerism (from the Greek *atropos*, "not turning") occurs when the rotation about a single bond is sterically hindered to the point that the individual conformers can be isolated.

Regardless of the type of chirality, if two chiral molecules are mirror images of each other, they are referred to as enantiomers or antipodes. If a molecule possesses multiple chiral centers, the number of possible chiral isomers is given by $2^k$, where $k$ denotes the number of asymmetric centers. In certain cases, molecules have less than this amount of distinct isomers, as some configurations may be superimposable, these are termed *meso* forms. When the configuration of one or more chiral centers differs between two molecules which are not mirror images, they are termed diastereomers. Figure 2 demonstrates these relationships using *cis*-permethric acid (see Section 1.4.2) as an example.

A compound containing one specific configuration of a chiral molecule is generally referred to as homochiral, or, depending on the type of isomer, enantiopure or diastereopure. A mixture of different configurations is referred to as heterochiral or scalemic, except in the case of a mixture containing equal amounts of two antipodes, which is referred to as a racemate (noun) or racemic (adjective). Note that "racemic mixture" and "racemic compound" are not interchangeable: the former simply denotes an equal ratio between two enantiomers, while the latter refers to materials with a particular crystallization phase diagram.

Figure 2: The configurations of permethric acid, illustrating the different types of optical isomerism. The top two structures are referred to as *cis*-permethric acid, the bottom two structures are referred to as *trans*-permethric acid.
Since enantiomers have nearly identical molecular structures (i.e. the number and type of atoms, bond lengths, bond energies etc.), their physical properties (such as melting/boiling point, density, etc.) are practically equal. However, chiral molecules have one distinguishing physical characteristic not found in other conformational isomers: their ability to rotate the polarization direction of plane-polarized light. This optical rotation was first described by Biot [9] and was later used by Pasteur [10] to identify the two enantiomers of sodium ammonium tartarate. Thus, chiral molecules have been termed "optically active", with the term "optical isomer" to refer to the different configuration of a given chiral compound. The ratio of different configurations in a mixture is generally referred to as "optical purity", homochiral molecules are referred to as "optically pure".

In an achiral environment, such as achiral solvents or reactions with achiral molecules, the behaviour of enantiomers is identical. However, their behaviour can differ significantly in chiral environments, such as chiral solvents, in contact with enzymes or biological systems (which contain numerous homochiral molecules, e.g. the essential amino acids, adrenaline, cholesterol, thyroxine, etc.). The differences between the effects of enantiomers on biological systems, especially the human body, is one of the main reasons why the separation of different configuration of chiral compounds, called chiral resolution (see Section 1.2), is a key subject in contemporary chemical engineering.

In some cases, variations in the effects of chiral molecules on biological systems may be benign, such as differences in olfactory perception. For example, the (R)-(+) isomer of the naturally occurring cyclic terpene limonene has a citrus-like fragrance, while its antipode, (S)-(−)-limonene is reported as having the aroma of pine or turpentine [11]. Carvone is a terpenoid (also naturally occurring) with differing fragrances: (−)-carvone has a spearmint-like aroma, while the smell of (+)-carvone is similar to caraway seeds [12].

Often, only one configuration of a chiral pharmaceutical compound enacts the desired biological effects, while the antipode is either less effective or completely inert. An example of this is seen with the antidepressant citalopram, a selective serotonin reuptake inhibitor that is marketed in racemic form, under the brand names Celexa and Cipramil. However, the efficacy of (1S)-(+) citalopram (referred to as escitalopram) is 40 times that of its antipode [13], thus pure escitalopram is also available, under the brand names Lexapro or Cipralex. Another example is the broad-spectrum antibiotic fosfomycin, which has only one active diastereomer, (1R,2S)-fosfomycin
In some cases, however, antipodes of pharmaceutically active chiral compounds may have harmful effects. Levofloxacin is a broad-spectrum antibiotic that, as its name suggests, is the \((S)-(-)\) enantiomer of the chiral molecule ofloxacin. The antipode, \((3R)-(+)\)-ofloxacin is not only less effective, it also carries an increased risk of severe side effects such as seizures \([15]\). Perhaps the most well-known case involving adverse effects from a chiral drug administered in racemic form is phthalimido glutarimide, commonly referred to as thalidomide. Marketed in West Germany under the brand name Contergan to mitigate the effects of morning sickness in pregnant women, it was responsible for severe limb deformities in several thousand newborns \([16, 17]\). Although it is suspected that \((3R)-(+)\)-thalidomide is responsible for the relief of nausea, and the \((S)-(-)\) enantiomer exerts the teratogenic effect, a definite conclusion could not be established due to the \textit{in vivo} racemization of thalidomide \([18]\). This incident led to much more stringent regulation of chiral compounds, and highlighted the importance of chiral resolution.

\subsection{Nomenclature of Chiral Compounds}

Since initially, chiral molecules could be distinguished solely based on their optical rotation, the earliest nomenclature is based on the direction in which they rotated the plane of polarization. If, from the viewpoint of an observer towards whom the light is travelling, the shift in polarization is clockwise, the molecule is designated \((+)\) or dexorotatory. Conversely, molecules rotating the polarization plane counter-clockwise are designated \((-)\) or levorotatory. Racemates are denoted by \((\pm)\).

The first method for representing the configuration of chiral molecules, independent of their rotation, was developed by \textsc{Fischer} \([19, 20]\). Based on this representation, a general system of nomenclature was proposed by \textsc{Rosanoff} \([21]\). Glyceraldehyde was chosen as a reference molecule and its dexo- and levorotatory forms were arbitrarily assigned the designations \textit{D-\((+)\)-glyceraldehyde} and \textit{L-\((-)\)-glyceraldehyde}, respectively. The designations originate from the Latin \textit{dexter} (right) and \textit{laevus} (left). Molecules which can be synthesized from \textit{D-\(\text{glyceraldehyde}\}} by steps that do not affect the chiral center are also assigned the \textit{D} descriptor and vice versa. Racemates are denoted by the descriptor \textit{DL-}. Because the designations are assigned by comparison to a reference molecule, the \textit{D/L} system is also referred to as relative configuration.

The current system of nomenclature for describing absolute configurations was
developed by Cahn, Ingold and Prelog [22] (later amended by Prelog and Helmchen [23]). The system involves ranking the four ligands attached to a chiral carbon based on atomic numbers, then assigning descriptors (R) or (S) based on the direction of rotation among the three top ranked substituents. The descriptors originate from the Latin words conveying the moral associations with right and left, rectus (honest, proper) and sinister (adverse, inappropriate). Racemates are denoted by (RS)-. A schematic demonstration of the nomenclature is provided in Figure 3.

![Figure 3: Assignment of absolute configuration descriptors. Numbers indicate substituent ranks according to Cahn–Ingold–Prelog priority rules [22].](image)

The R/S descriptors can include the position of the chiral center (as assigned by the IUPAC rules for numbering atoms [24]), written as (2R)-. Since the descriptors are specific to one chiral center, a descriptor must be supplied for each stereogenic atom in the molecule, in order of increasing positions, e.g. (1R,3S)-. If only the relative configurations of chiral centers are known, the chiral center in the lowest position is described as (R*)- and subsequent centers are assigned (R*)- or (S*)- indicating identical or opposite configurations, respectively, e.g. (1R*,3R*)- [25, p. 1963, ST-6].

Because of their largely different definitions, there are no fixed relationships between the +/−, D-/L- and R/S systems. Molecules with either D- or L- descriptors, and molecules of any combination of R and S centers may be dexorotatory or levorotatory (although optical rotation can be calculated from the absolute configuration by numerical computations [26]). The D-/L- and R/S descriptors often correlate, e.g. almost all essential amino acids are L-(S)- enantiomers. Cysteine is a noted exepction, however: the proximity of the sulphur atom (ranked higher in the CIP system than carbon, oxygen or nitrogen) to the chiral center causes a change in its absolute configuration descriptor, making it a relatively rare L-(R)- enantiomer. For this reason, the D-/L- system is still used in a number of areas (such as carbohydrates or amino acids), as these designators are unaffected by the introduction of heteroatom-bearing sidechains.
1.1.2. Quantification of Chirality

Complex mixtures of chiral compounds, such as those containing more than two distinct optical isomers, are described in the usual manner, e.g. by specifying percent compositions or the ratios between the isomers. However, for mixtures of two enantiomers or diastereomers of a given chiral compound, a special method of quantification is defined as follows.

Let $Q$ denote the "quantity" of a given compound, either a physical quantity such as mass or number of moles, or some quantity in linear correlation with a physical quantity such as the detector signal of an analytical instrument. Furthermore, let the indices maj and min refer to the quantities (as previously defined) of the major component (the component in excess) and the minor component, respectively. If the components are enantiomers, the so-called enantiomeric excess (ee) is defined by the following equation.

$$
\text{ee} = \frac{Q_{\text{maj}} - Q_{\text{min}}}{Q_{\text{maj}} + Q_{\text{min}}}
$$

For diastereomers, the definition of the analogous diastereomeric excess (de) is formally identical to Eq. 1.1, the only difference being that $Q$ refers to the quantity of diastereomers.

The values of both ee and de range from 0, in the case of a racemate, to 1 for pure enantiomers or diastereomers. To avoid ambiguity, the major component is typically specified along with the value, e.g. $\text{ee} = 0.8 \, (R)$. For the majority of compounds (including those discussed in this thesis), there is a linear correlation between ee/de and optical rotation (which is zero for racemates and maximal/minimal for enantiomer/diastereopure samples), thus the values of ee or de are equal to normalized absolute values of optical rotation. This linear correlation, however, is not universal, as some compounds present a nonlinear relationship between ee/de and optical rotation due to the Horeau effect.

1.2. Chiral Resolution

The first chiral resolution was performed by Pasteur [10] after studying the crystals of sodium ammonium tartarate under a microscope, and observing two distinct crystal shapes which were mirror images of each other. After manually sorting the different crystals, the two groups were found to have opposing optical rotations, in-
indicating that a separation of enantiomers had in fact taken place.

Sodium ammonium tartrate can be resolved by mechanical separation of enantiomeric crystals because homochiral molecular interactions are stronger than heterochiral ones, resulting in crystalline phases containing only one of the enantiomers (referred to as conglomerates). However, this property is fairly rare, as in a majority of substances, heterochiral interactions are stronger than homochiral ones, resulting in so-called racemic compounds (crystalline phases of racemic composition). In a small number of cases, homo- and heterochiral interactions are roughly equal, resulting in solid solutions (crystalline phases of variable composition). In all cases, chiral resolution is performed by processes which are said to be stereoselective, i.e. capable of distinguishing between different stereoisomers.

One commonly used approach for chiral resolutions is based on diastereomer crystallization, in which the racemate is reacted with a homochiral auxiliary, the so-called resolving agent. The ratio of these, referred to as the molar ratio (mr), is calculated from the molar quantities (n) of the resolving agent and the racemate (indices res and rac, respectively) by the following equation:

\[
\text{mr} = \frac{n_{\text{res}}}{n_{\text{rac}}}
\] (1.2)

Assuming a 1:1 stoichiometric ratio between for the diastereomer formation, a molar ratio of 1 corresponds to equivalent amounts of racemate and resolving agent. This is fairly typical, and further examples will be presented with this assumption.

In the most basic approach, the resolving agent is reacted with the racemate in equivalent amounts, forming two diastereomers. If these differ sufficiently in their physical characteristics, they may be separated by fractional crystallization. This approach may be represented with the following schematic reaction equation, in which R and S denote the optical isomers of the racemate, A denotes the resolving agent and ↓ denotes precipitated compounds.

\[
\text{RS} + 2A \rightarrow \text{R-A}↓ + \text{S-A}
\]

An alternative approach, proposed by Pope and Peachey [27], involves adding the resolving agent only in half-equivalent ratio (which, for a 1:1 stoichiometric ratio, corresponds to mr = 0.5), with an achiral auxiliary (denoted by X) that prevents precipitation of the non-crystallizing form of the racemate. The resolving agent reacts with one stereoisomer of the racemate preferentially, which is enriched in the crystalline phase, leaving behind a mother liquor enriched in the opposite isomer.
Schematically, this is represented by the following equation (for clarity, a complete separation of stereoisomers R and S is shown, rather than the partial separation described previously).

\[ RS + A + X \rightarrow R-A\downarrow + S-X \]

The achiral auxiliary can be structurally related to the resolving agent, or a mineral acid such as hydrochloric acid. A modified version of the Pope–Peachy resolution method omits the achiral auxiliary altogether, relying on the solubility difference between the diastereomer and the unreacted isomers:

\[ RS + A \rightarrow R-A\downarrow + S \]

A recent development, first reported by Vries et al. [28], involves using multiple, structurally related resolving agents, referred to as Dutch resolution. Several reports [29–31] have indicated that the resolution is effected by a combination of resolving agents which are present in the crystalline phase in different ratios. Typically, a large number of resolving agents are applied to a given racemate, not all of which are necessarily effective.

In diastereomer formation-based resolutions, the key step can either involve the formation of diastereomeric salts or molecular complexes, or the formation of covalent compounds between the resolving agent and the racemate. Compounds successfully resolved using this latter method include 2-bromo-2,3-dihydro-1H-cyclopenta[a]naphtalen-1-ol (an indanol-type haloalcohol) by diastereomer crystallization after esterification with (S)-(+)\:-2-(6-methoxynaphthalen-2-yl) propanoic acid (naproxen) [32]. Another example, as well as a case where mr = 1 does not correspond numerically to the equivalent amount, is the resolution of trans-1,3-diphenyl-2,4-bis-[α-hydroxybenzyl]-cyclobutane with O-acetyl mandelic acid. Since the racemate has two virtually identical hydroxyl groups available for esterification, the resolution is performed with the equivalent amount, i.e. \( mr = 2 \) [33].

Besides diastereomer crystallization, another common approach for the separation of optical isomers is kinetic resolution. In this technique, a racemate is reacted with a (typically achiral) substrate, using a chiral catalyst. Owing to its chirality, the catalyst interacts differently with the optical isomers of the racemate, which are thus converted at differing reaction rates. If the two rates are sufficiently dissimilar, the reaction product will contain one isomer in almost enantiopure form, leaving its antipode almost completely unreacted.

The first kinetic resolution was reported by Pasteur [34], having observed that a
racemic solution of ammonium tartarate, upon microbial fermentation, became lev-
orotatory. The fermentation process consumed \((R,R)\)-tartaric acid at a much higher rate than its antipode, leading to an enrichment of \((S,S)\)-\((-\)\)-tartaric acid in the mother liquor.

Enzymes are often not only chemo- but stereoselective catalysts as well, and as such, they are often used for kinetic resolution [35]. The first "synthetic" (i.e. non-biological) kinetic resolution has been reported by MARCKWALD et al. [36], in which \((-\)\)-menthol was observed to undergo esterification with \((-\)\)-mandelic acid faster than with its antipode. Although not as widespread as enzymatic kinetic resolution, this approach is nonetheless an active area of research [37].

Chiral resolution can be accomplished by a chiral chemocatalytic step. Typically, the chemocatalysts used for these approaches are coordination complexes with chiral ligands, which can be applied to either homogeneous or heterogeneous catalytic techniques [38]. Chirality in these catalysts can be present at heteroatoms, as is the case with so-called P-chiral catalysts, containing chiral phosphorous atoms [39]. Furthermore, ligands may not be chiral at all, with chirality being present only at the metal coordination center, e.g. ruthenium(II) [40].

An inherent limitation of kinetic resolution, whether enzymatic or not, is the theoretical maximum yield of 50% with respect to the racemate, i.e. the fact that one isomer is left behind unreacted. The so-called dynamic kinetic resolution approach eliminates this shortcoming by combining kinetic resolution with an \textit{in situ} racemization, constantly converting the unreacted isomer into racemate, thereby raising the theoretical maximum yield to nearly (but never exactly) 100%. Numerous enzymatic and non-enzymatic methods are discussed in the reviews by PELLISSIER [41, 42].

### 1.3. Supercritical Fluids

A compound above its characteristic critical pressure and temperature is said to be in the supercritical state, or referred to as a supercritical fluid. The critical pressure and temperature define the so-called critical point. This is illustrated on a generalized pressure–temperature phase diagram in Figure 4. It must be noted that when discussing supercritical fluids, it is assumed that both the pressure and the temperature are relatively close to the critical values. For example, nitrogen and oxygen are both above their respective critical temperatures \((-146.9\, ^\circ\text{C}\) and \(-118.6\, ^\circ\text{C}\)) under atmospheric conditions. However they are not referred to as supercritical when pressurized, since their absolute temperature under standard conditions is roughly twice
the critical value. For comparison, supercritical carbon dioxide is routinely used at as little as 5–10 °C above its critical temperature.

The physical properties of a material in its supercritical state are between those of the material in its gaseous and liquid state. Most notably, the density of supercritical fluids is closer that of the liquid state, while its viscosity more closely resembles that in the gaseous phase. On the molecular level, this is explained by the formation of supramolecular agglomerates of roughly liquid-like structure, which fill out the available volume as gases would. As a consequence, supercritical fluids also have no surface tension.

Supercritical fluids have many desirable properties from an industrial viewpoint. Chief among these is their tunability: certain physical properties of supercritical fluids vary sensitively with the pressure and temperature, especially near the critical point. Thus, for example, the density and solvent power of a supercritical fluid can be precisely varied. Another advantage is their facile removal from solutions: by dropping the pressure below its critical value, the supercritical fluid becomes gaseous (as long as the temperature is maintained above critical), leading to any materials dissolved therein precipitating with very little contamination.

Due to its advantageous properties, carbon dioxide is a widely applied supercriti-
Supercritical fluids are extremely effective as extraction media, as their gas-like viscosity and lack of surface tension enables them to penetrate porous materials to a large degree, while their liquid-like density allows the dissolution of large quantities of solute. Supercritical acetone (at 240 °C and 60–65 bar) has been applied to the extraction of oriental beech (Fagus orientalis) fatty acids, yielding a product rich in linoleic acid [43]. Using radioisotope labelling, supercritical methanol extraction was shown to outperform high-temperature distillation for the removal of pesticide residues from soil or plant samples [44].

Because of their tunable properties, supercritical fluids have also attracted interest as reaction media. The upgrading of pyrolysis bio-oil from rice husk, catalyzed by HZSM-5 zeolite (which cracks heavy components and facilitates esterification), was shown to proceed more efficiently in supercritical ethanol compared to subcritical ethanol [45]. The ammonothermal synthesis of gallium nitride single crystals was realized using supercritical ammonia (at 400 °C and 2400 bar) [46].

Supercritical water finds great use as a reaction medium, in part due to its decreased polarity. Aqueous wastewater effluents, when heated and compressed past the critical point of water (374 °C and 221 bar), become miscible with gaseous oxygen, allowing the oxidation of contaminants in a homogeneous-phase reaction. This method of treatment is known as supercritical water oxidation (SCWO) [47]. Other reactions carried out in supercritical water include the conversion of methane to methanol [48] and the hydrothermal synthesis of various metal oxide nanoparticles [49].

The low viscosity and tunability of supercritical fluids also led to their application as mobile phases in chromatographic separations, termed supercritical fluid chromatography (SFC). The technique is widely applied to pharmaceutical compounds, numerous examples are included in the review by De Klerck et al. [50]. Supercritical fluid chromatography has also been implemented in preparative scale, examples of enantiomer separations carried out by this technique have been reviewed by Miller [51].

1.3.1. APPLICATIONS OF SUPERCRITICAL CARBON DIOXIDE

Apart from the advantageous properties of supercritical fluids described earlier, supercritical carbon dioxide (scCO₂) in particular has several additional benefits that...
make it attractive for industrial applications. Its critical pressure is 73.8 bar, relatively 
low compared to the critical pressures of other materials, while its critical temperature 
of 31.0 °C allows its use for heat-sensitive compounds such as biological or thermo-
labile materials. It is abundantly and economically available, and if biogenic – e.g. 
harnessed as the byproduct of yeast fermentation – it does not contribute to increas-
ing carbon dioxide levels in the atmosphere. It is a non-flammable, non-explosive 
substance that is non-toxic if it contaminates products in trace amounts.

One of the earliest applications of scCO$_2$ was the extractive decaffeination of cof-
fee and tea [52, p. 294]. Extraction of plant materials continues to be a major ap-
plication of supercritical carbon dioxide. Examples include the extraction of hops 
[53], the extraction of fatty acids from various microalgae [54] and from chia (Salvia 
hispanic L.) seeds [55], as well as the extraction of carotenoids, tocopherols and sito-
sterols from industrial tomato by-products [56]. The scale-up of extraction processes 
with scCO$_2$ has also been accomplished: a 2009 survey by Gamse [3] cites 59 indus-
trial scale extraction plants operating worldwide, with a combined capacity of over 
200 000 tonnes annual capacity.

Extractive applications of supercritical carbon dioxide for purposes other than 
treating plant materials have also been developed. Examples include the extraction 
of compounds causing wine taint from cork stoppers [57], aerogel drying [58], the 
removal of residues from etched semiconductors [59] or soil decontamination [60].

There are several examples of scCO$_2$ being used as a reaction medium, as high-
lighted in the review by Han et al. [4]. Selective free radical reactions of alkanes 
show increased reaction rates in near-critical and supercritical carbon dioxide [61]. 
Combining scCO$_2$ with ionic liquids is an area of considerable research interest, it has 
been applied for example to the synthesis of the antimicrobial agent carvacrol [62].

The possibility of using scCO$_2$ as a reaction medium for enzymatic reactions has 
been first demonstrated in 1985, when certain enzymes were found to be stable in 
scCO$_2$ [63–65]. Since then, numerous enzymatic reactions have been realized in 
scCO$_2$, such as biodiesel production using Candida rugosa and Rhizopus oryzae lipases 
[66] or the esterification of lactic acid using Candida antarctica lipase B (Novozyme 
435) [67].

Various crystallization and particle formulation processes utilize scCO$_2$ as a sol-
vent or antisolvent. An overview of these technologies is provided in the reviews by 
Jung et al. [5] and by Reverchon [68]. Major techniques are summarized below.

scCO$_2$ is used as a solvent in the approach known as rapid expansion of super-
critical solvent (RESS). This process involves dissolving the desired material in supercritical CO$_2$, followed by the expansion of the solution through a nozzle. During the expansion, the pressure of carbon dioxide drops below the critical value, causing it to change into the gas state. This leads to a rapid nucleation of the dissolved components, potentially yielding a product with extremely narrow particle size distribution. This approach is advantageous from an environmental point of view as it utilizes scCO$_2$ as the sole solvent. However, the requirement that the materials be soluble in scCO$_2$ restricts the types of compounds that can be crystallized using this method.

Several techniques utilize carbon dioxide as an antisolvent. Generally, these methods involve contacting the materials dissolved in an organic solvent with supercritical carbon dioxide, which decreases the solvent power of the organic solvent and leads to the precipitation of the solutes. The gas antisolvent process (GAS) involves loading the solution into a precipitation vessel and pressurizing it with scCO$_2$. In the SAS method, the solution is injected into a precipitation vessel maintained under pressure by a flow of scCO$_2$. The GAS and SAS methods are described in more detail in Section 2.3.4 (GAS) and Section 2.4 (SAS). Another antisolvent technique, called solution-enhanced dispersion by supercritical fluids (SEDS), consists of pulverizing the organic solution and scCO$_2$ through two coaxial nozzles, using scCO$_2$ not only as an antisolvent but also as an aid in mechanically dispersing the organic solution. These methods can be utilized for a wider range of solutes as the RESS process, since the organic solvent most appropriate for the solute can be chosen. However, the use of organic solvents makes these technologies less environmentally favourable.

Instead of being used as a solvent or antisolvent, scCO$_2$ can be solubilized into suspensions or melts of the target materials. This technique is known as particles from gas-saturated solutions/suspensions (PGSS) and is often used with polymers, which can absorb large amounts of CO$_2$ while swelling and/or melting significantly below their melting point or glass transition temperature. The resulting so-called gas saturated solution/suspension is then expanded through a nozzle, resulting in particle formation.

The above mentioned techniques are applied in a wide variety of fields for composite crystallization or microencapsulation. Typically, these processes involve the dissolution of multiple substrates, e.g. a pharmaceutical compound and the encapsulating polymer, then submitting them to one of the supercritical precipitation methods. As an example, the water solubility of rosemary leaf ethanol extracts can be improved
by encapsulation with poloxamers using the SAS technique [69].

The tunable properties of scCO₂ can afford control over the properties of the crystallized particles, most importantly the particle size. One example of such control was realized using the GAS technique, producing lysozyme, insulin and myoglobin particles with sizes varying from 0.05–2.0 μm [70]. Supercritical precipitation methods have been successful in controlling the polymorphism of the crystallized materials, e.g. during the recrystallization of caffeine [71].

Other uses of scCO₂ include enhanced oil recovery (EOR) [72], the working medium in solar Rankine cycles [73] and impregnation of wood with biocides [74]. Applications of scCO₂ in non-technical areas such as dry cleaning have also been patented [75].

The possibility of chiral resolution with scCO₂ via selective extraction of enantiomers, was first reported in 1994 by Fogassy et al. [76]. The first reported results were published in 1997 by Simándi et al. [77]. The resolution process involves the addition of half-equivalent quantities of a resolving agent to a racemate in an organic solvent, evaporating the organic solvent and separating the diastereomeric salts from the unreacted enantiomeric mixture by the extraction of the latter with scCO₂. This technique was successfully applied to the resolution of several compounds, such as ibuprofen [77–79], cis- and trans-permethric acids [77, 80], 6-fluoro-2-methyl-1,2,3,4-tetrahydroquinoline [81], trans-2-chloro-cyclohexan-1-ol [82], N-methylamphetamine [83], trans-1,2-cyclohexanediol [84, 85] as well as camphorsulfonic, phenylpropionic and mandelic acids [86].

Supercritical fluid chromatography can also be applied to the separation of enantiomers, a review by Terffloth [87] gives an overview of the technology. In addition to regular stationary phases, the use of molecularly imprinted polymers has also been investigated [88].

### 1.3.2. Antisolvent Resolution

Although widely used for encapsulation or crystallization, supercritical carbon dioxide antisolvent techniques have not been extensively applied to the separation of enantiomers. The earliest reported result, by Kordikowski et al. [6], describes the resolution of ephedrine with mandelic acid using the SEDS process. A detailed study of the effects of temperature and pressure was carried out between 100–300 bar and 35–75 °C using methanol, and it was found that the resolution is influenced by the density and the temperature of the supercritical phase. Diastereomeric excesses in
the produced salts ranged between 0.81–0.86, significantly above the value of 0.76 obtained via conventional resolution experiments (dissolution in boiling ethanol and crystallization by cooling the solution). Furthermore, while diastereomers obtained via conventional methods required two recrystallizations to achieve purities of > 99% (antipode not detectable by capillary electrophoresis), particles produced by SEDS achieved this purity after only one recrystallization. However, the morphology of the produced crystals was the same for both methods.

The resolution of mandelic acid with 1-phenylethylamine has been reported by Martín et al. [7] using the SAS method. The effects of pressure and temperature were studied using three approaches: using equivalent or half-equivalent amounts of the resolving agent and feeding the racemate–resolving agent solution (ethyl acetate–DMSO) into a precipitator pressurized with scCO₂, as well as feeding a solution of mandelic acid (also ethyl acetate–DMSO) into the precipitator first, followed by a solution of 1-phenylethylamine (in half-equivalent amount). This last approach afforded the best results at 80 bar and 55 °C, yielding particles with a diastereomeric excess of 0.63, with 92% of (R)-(−)-mandelic acid recovered as particles.

A direct precursor to the work presented in this thesis, the resolution of ibuprofen using 1-phenylethylamine has been reported by Santarossa et al. [8], using the GAS and SAS processes. Particles were only obtained with the SAS process between 100–120 bar and 40–50 °C from an ethyl acetate–DMSO solution, however, these experiments suffered from either low recovery of ibuprofen (< 10%) or low diastereomeric excess (< 0.20). No experiment yielded a diastereomeric excess of more than 0.40. Another approach was also investigated, in which an ethyl acetate solution of 1-phenylethylamine was injected into a precipitation vessel along with an ibuprofen-saturated scCO₂ stream. Between 95–125 bar and 35–50 °C, these experiments could achieve up to 25% recovery of ibuprofen in the particles, however, diastereomeric excesses remained below 0.40.

1.4. Investigated Racemates

1.4.1. Ibuprofen

Ibuprofen (abbreviated as IBU) is an accepted trivial name (derived from the names of the isobutyl, propanoic acid and phenyl moieties) for 2-[4-(2-methylpropyl)phenyl]propanoic acid. The second carbon atom of the propanoic acid subgroup is chiral, resulting in the two enantiomers shown in Figure 5.
Figure 5: Structure and configuration of (R)-(−)-ibuprofen and (S)-(+)−ibuprofen.

Ibuprofen has significant pharmaceutical uses, belonging to a category of compounds termed non-steroidal anti-inflammatory drugs (NSAIDs). Developed and patented in 1962 by Boots Corporation [89], it was sold originally under the trade name Brufen. It was approved as a prescription drug in the United Kingdom and in the United States in 1969 and 1974, respectively, and became available over the counter in the UK in 1983 and in the US in 1984 [90].

Most commonly, ibuprofen is used as an analgesic and antipyretic, i.e. to relieve pain and fever, as well as treatment of various rheumatoid diseases. Other uses include the management of primary dysmenorrhea [91] and the medical condition known as patent ductus arteriosus, in which a channel between the aorta and the pulmonary artery (the ductus arteriosus, normally present in the fetal stage) fails to close after birth, causing circulation problems [92]. Other potential uses of ibuprofen include the treatment of Alzheimer's disease [93] or as a preventive measure against oral cancer [94].

Arylpropionic acid NSAIDs, such as ibuprofen (along with, for example, naproxen or flurbiprofen), are often marketed in racemic form despite only one of the enantiomers being pharmaceutically relevant [95, p. 56]. In the case of ibuprofen, the desired biological effect is enacted by (S)-(+)−ibuprofen [96], also referred to as dexibuprofen (denoting the dextrorotatory nature of the enantiomer). Racemic formulations of ibuprofen are effective because (R)-(−)-ibuprofen undergoes stereoselective bioinversion within the body [97–99], however, when administered in enantiopure form, the bioavailability of (S)-(+)−ibuprofen increases by a factor of 100 [98]. Furthermore, during the bioinversion process, (R)-(−)-ibuprofen can engage in acyl exchange with naturally occurring triglycerides, resulting in the accumulation of ibuprofen residues in fatty tissue [95, pp. 56–58]. Therefore, ibuprofen was one of the first candidates for the so-called "chiral switch" [100], in which racemic formulations of chiral pharmaceuticals are phased out in favor of single-enantiomer products.
Originally, ibuprofen was synthesized using the Boots process [89], which was later supplanted by the more efficient Hoechst process [101]. In both processes, the initial step is the Friedl–Crafts alkylation of isobutylbenzene. In the Hoechst process, this step is followed by a catalytic hydrogenation and a catalytic carboxylation to arrive at the final product. Although successfully implemented on the industrial scale, these processes yield racemic ibuprofen, thus obtaining the more effective (S)-(+)–ibuprofen requires an additional resolution step.

As an alternative to resolving racemic ibuprofen, asymmetric syntheses selective to (S)-(+)–ibuprofen have been developed. These approaches typically use achiral feedstocks, inducing chirality in an asymmetric reaction step. Examples of these steps include lipase-catalyzed asymmetric acylation of a diol precursor [102] or stereoselective hydrogenolysis of an epoxide intermediate [103]. Alternatively, resolution of a racemic chiral intermediate could be carried out, e.g. by dynamic kinetic resolution with (S)-(+)–lactic acid amides [104].

In addition to the asymmetric synthetic routes, numerous approaches for the resolution of ibuprofen have been developed, a non-exhaustive review of which is presented here. The separation of ibuprofen enantiomers has been achieved on a preparative scale by chromatographic methods [105], including simulated moving bed chromatography [106]. The traditional, crystallization-based chemical resolution of ibuprofen was successful using (S)-(−)-phenylglycinol as a resolving agent [107], affording the (S,S)-salt with a diastereomeric excess of 53%.

The separation of ibuprofen enantiomers via supercritical fluid extraction was initially reported by Simándi et al. [77] using (R)-(−)-1-phenylethanamine as the resolving agent. A detailed investigation of parameter effects was carried out by Keszei [108], while a study of possible resolving agents was conducted by Valentine [109]. Further research into the ibuprofen–(R)-(−)-1-phenylethanamine resolution system [78, 79] was the precursor to the research into the resolution of ibuprofen presented in this thesis.

Classical liquid extraction methods are also viable for the enantioseparation of ibuprofen, using various resolving agents, such as L-tartaric acid [110] or hydroxypropyl-β-cyclodextrin [111]. The enantioselective adsorption of ibuprofen enantiomers in metal-organic frameworks has also been reported [112].

Several enzymatic methods for resolving ibuprofen have been reported, such as using immobilized lipases of Rhizomucor miehei for a selective ester cleavage [113]. The separation of the enzymes from the reaction mixture can be facilitated by anchor-
ing them onto magnetic particles, this method has been used to immobilize bovine serum albumin [114] and *Candida rugosa* lipase [115]. Another approach for improving the separation of the enzymes from the products is the use of ionic liquids. *Naik et al.* [116] described a resolution in which ibuprofen was anchored onto a specially functionalized ionic liquid, and a *Candida antarctica* lipase was used to selectively cleave (S)-(+)‐ibuprofen from the anchor.

Enzymatic separations of ibuprofen combined with membrane technology have also been reported. The *Candida rugosa* Type VII lipase was incorporated into a monolithic membrane, increasing the transport of (S)-(+)‐ibuprofen through it, enabling the separation of enantiomers to be carried out via microfluidic filtration [117]. Membrane reactors have been utilized in the kinetic resolution [118] or dynamic kinetic resolution [119] of ibuprofen (both processes used *Candida rugosa* lipases). In another example, pervaporation was used to remove the water from the reaction mixture during stereoselective esterification of ibuprofen with a *Candida rugosa* lipase [120].

### 1.4.2. cis-Permethric Acid

Permethric acid, without any qualifiers, refers to 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid. The cyclopropane ring contains two chiral carbon atoms, resulting in four possible configurations, see Fig. 2 on p. 5. The pair of configurations in which the sidechains are on the same side of the cyclopropane ring are referred to as cis-permethric acid (abbreviated cPA). As shown in Figure 2, these molecules are in fact mirror images of each other and therefore constitute a pair of enantiomers. Figure 6 shows the structure and abbreviations of these two enantiomers. The other two configurations, referred to as trans-permethric acid (tPA), are also enantiomers, however, the relationship between the cis- and trans- forms is diastereomeric.

\[
\begin{align*}
\text{Cl} & \quad \text{Cl} & \quad \text{O} & \quad \text{OH} \\
\text{Cl} & \quad \text{Cl} & \quad \text{O} & \quad \text{OH}
\end{align*}
\]

\((-\text{cPA}) \quad (+\text{cPA})

*Figure 6:* Structure and configuration of (1S,3S)-(−)-*cis*-permethric acid and (1R,3R)-(+)-*cis*-permethric acid.
The first reported use of permethric acid was published by ELLIOTT et al. [121], describing analogues of naturally occurring pyrethroid insecticides in which the dimethylvinyl sidechain of chrysanthemic acid was substituted for a dichlorovinyl moiety. They reported two derivatives of permethric acid being effective as pesticides: the 3-phenoxybenzyl ester (permethrin) and the 5-benzyl-3-furylmethyl ester (resmethrin). Both compounds were found to be 2 orders of magnitude more effective than allethrin, possessing low mammalian toxicity. Additionally, it was reported that the efficacy of (R,R)-permethrin was higher than the racemic ester mixture. Since its initial use, other esters of permethric acid have been used as pesticides [122], while its amides were found to possess larvicidal properties [123].

Although permethric acid is not used directly for pesticidal purposes, it is a key intermediary in the syntheses of several pesticidal compounds such as permethrin (see above). Since it is often the only chiral moiety, its synthesis or resolution is the method by which chirality is introduced into these pesticidal formulations. It is also produced as the photolytic residue of permethrin [124], β-cyfluthrin [125] as well as cypermethrin and decamethrin [126] and the metabolite of cypermethrin [127], β-cyfluthrin [128] and permethrin [129].

In discussing the syntheses of cPA, an important distinction must be made regarding stereoselectivity: since permethric acid has four possible isomers, synthetic routes selective to cis-permethric acid are stereoselective (i.e. favoring cPA over tPA) even if the produced cPA is racemic. Therefore the syntheses presented below will be described as "stereoselective" if they are selective to cPA rather than tPA, and "enantioselective" if they exhibit selectivity towards one enantiomer of cPA. The syntheses presented below are summarized in Table 1 (see p. 24) according to these two categories.

Permethric acid was first synthesized by FARKAŠ et al. [130] by reacting 1,1-dichloro-4-methyl-1,3-pentadiene with ethyl diazoacetate to produce the ethyl ester of cPA, see Figure 7. However, due to the inherent risks posed by working with ethyl diazoacetate, subsequent synthetic strategies (both symmetric and asymmetric) avoided this method. Although various synthetic routes to cPA have been published or patented [131], the formation of the characteristic dimethylcyclopropane ring is typically accomplished by one of three approaches.

The first of these approaches is the so-called Favorskii rearrangement, shown in Figure 8. By subjecting 2-chloro-3,3-dimethyl-4-(2,2,2-trichloroethyl)cyclobutanone to a strong base, 3-(2,2,2-trichloroethyl)-2,2-dimethylcyclopropanecarboxylic acid is
obtained, which is transformed to cPA by elimination of hydrochloric acid induced by potassium hydroxide. Using this method, MARTIN et al. [132] reported the synthesis of a 80:20 mixture of cPA and tPA, later developing the method to be exclusive to cPA [133]. By resolving the NaHSO₃ adduct of the cyclobutanone intermediate using 1-phenylethanamine, GREUTER et al. [134] achieved an enantioselective synthesis of (+)-cPA.

The dimethylcyclopropane ring can also be obtained by the intramolecular cyclization of 4,6,6,6-tetrachloro-3,3-dimethylhexanoic acid derivatives in the presence of sodium hydride, shown in Figure 9. This method was used by KLESCHICK [135] to achieve a stereoselective synthesis, obtaining a 85:15 ratio of cPA and tPA. The addition of an isopropyl sidechain to the oxazolidinone moiety causes steric interaction with the chiral chlorine atom that undergoes elimination, resulting in 92% pure (+)-cPA (albeit with 6% tPA impurities) [136].
Finally, the dimethylcyclopropane unit can be prepared by cleaving the heterocycle of a 3-oxabicyclo[3.1.0]hexan-2-one moiety (effectively a $\gamma$-butyrolactone ring condensed onto the dimethylcyclopropane group) via reduction with zinc and acetic acid, as shown in Figure 10. The bicyclic structure can be derived from the intramolecular cyclization of a diazo compound, this approach was first used by Kondo et al. [137] in a stereoselective synthesis affording cPA exclusively (tPA could not be detected by GC). The synthesis can be made enantioselective by resolving the chiral intermediate 1,1,1-trichloro-4-methyl-3-penten-2-ol, either by asymmetric derivatization [138] or via selective cleavage of the acylated alcohol with porcine liver acetone powder (PLAP) [139]. In both cases, (+)-cPA was obtained with ee = 0.98. Alternatively, the fused heterocyclic structure can be produced from naturally occurring (+)-$\Delta^3$-carene, resulting in an inherently enantioselective synthesis affording (+)-cPA with ee = 0.9 [140] or as high as ee = 0.98 [141].

The pH-driven resolution of racemic cis-permethric acid using (S)-(+)2-(N-benzylamino)butan-1-ol (see Section 1.5.2) as the resolving agent was described by Fogassy et al. [142] (also patented [144]), with follow-up publications on upgrading the purity of non-racemic mixtures of cPA [145] and the conformational flexibility of the resolving agent [146]. By treating a mixture of the sodium salt of cis-permethric acid

<table>
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<th>Key step</th>
<th>Stereoselective</th>
<th>Enantioselective</th>
<th>Figure</th>
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<td><em>Favorskii</em> rearrangement</td>
<td>MARTIN <em>et al.</em> [132]</td>
<td>GREUTER <em>et al.</em> [134]</td>
<td>8 (p. 23)</td>
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<td>MARTIN [133]</td>
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<td>Intramolecular cyclization</td>
<td>KLESCHICK [135]</td>
<td>KLESCHICK <em>et al.</em> [136]</td>
<td>9 (p. 23)</td>
</tr>
<tr>
<td>Heterocycle cleavage</td>
<td>KONDO <em>et al.</em> [137]</td>
<td>HATCH <em>et al.</em> [138]</td>
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<td>FISHMAN <em>et al.</em> [139]</td>
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<td>KLEMMENSEN <em>et al.</em> [140]</td>
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<td>MANDAL <em>et al.</em> [141]</td>
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Table 1: Summary of synthetic strategies for *cis*-permethric acid.
Figure 11: Two-step, pH-controlled resolution of *cis*-permethric acid with (*S*)-(*+*)-2-(*N*-benzylamino)butan-1-ol [142].

Figure 12: Resolution of *cis*-permethric acid with carene derivatives [143].
and (S)-(+)\,-\,(N\,-\text{benzylamino})\,-\text{butan-1-ol}} hydrochloride alternately with sodium hydroxide and hydrochloric acid, both enantiomers of cPA can be precipitated as their respective (S)-(+)\,-\,(N\,-\text{benzylamino})\,-\text{butan-1-ol} salts, as shown in Figure 11. Although the salts precipitate in "optically pure" form [142, p. 1386], an obvious drawback of this approach (as indicated by Fig. 11) is an inherent maximum yield of 50% with respect to the total racemic cis-permethric acid.

Another crystallization-based resolution technique, using carene-derived amines as resolving agent was described by PoPOV et al. [143]. A schematic of the resolution method is shown in Figure 12. Using 3,4,4-trimethyl-3b,4,4a,5-tetrahydro-2H-cyclopropa[3,4]cyclopenta[1,2-c]pyrazole (structure a in Fig. 12) as the resolving agent, the purities of the recovered cPA enantiomers were reported as $\geq 98\%$ for (−)-cPA and 95% for (+)-cPA. The (−)-cPA-containing diastereomer was recrystallized twice from methanol, the (+)-cPA-containing diastereomer was recrystallized three times (benzene–methanol 1:1, then methanol twice). The overall yield for both diastereomers was reported as 82–86%.

The resolution of cis-permethric acid with (R)-(+)\,-\text{1-phenylethanolamine} by supercritical CO$_2$ extraction was reported by SimáNDI et al. [80]. The diastereomers were formed by mixing organic solutions of cPA and half mole equivalent (R)-PhEA, then evaporating the solvent under vacuum. The resulting solid mixture was loaded into a packed column and extracted with supercritical carbon dioxide, to separate the unreacted cPA enantiomers from the diastereomeric salts. Values of ee were reported as 0.56 for (+)-cPA and 0.75 for (−)-cPA, with 81% recovery of cPA.

1.5. RESOLVING AGENTS

1.5.1. 1-PHENYLETHANAMINE

The structures of both the R and S configurations of 1-phenylethanolamine (abbreviated PhEA) are shown in Figure 13. cis-Permethric acid was resolved using (R)-PhEA, while the resolution of ibuprofen was investigated using both (R)-PhEA and (S)-PhEA.

Optically active 1-phenylethanolamine is used primarily as a resolving agent in the salt-based resolutions of acidic racemates. Numerous studies have been published on its use in the resolution of mandelic acid [147–149] or its derivatives [150]. It has also been applied to the resolution of essential amino acids, such as tryptophane [151], valine and phenylalanine [152]. InGERSOLL [153] described a method for the mutual resolution of 1-phenylethanolamine with malic acid, in which both compounds
could be obtained in enantiopure form. The acidic intermediates in the synthesis of (S)-3-amino-N-cyclopropyl-2-hydroxyalkanamides, potent inhibitors of the Hepatitis C virus, have also been resolved using (R)-(+)1-phenylethanol.

Figure 13: Structure and configuration of (R)-(+)1-phenylethanolamine and (S)-(−)-1-phenylethanolamine.

In addition to its use as a resolving agent, the enantiomers of 1-phenylethanolamine are also used as chiral building blocks for various molecules. (R)-(+)1-Phenylethanolamine has been used in the construction of chiral ionic liquids [154] or in the synthesis Jaspine B (also called pachastrissamine), a d-ribo-phosphatidylcholine derivative with strong cytotoxicity against melanoma cells [155]. (S)-(−)1-Phenylethanolamine has been used to produce artificial sweeteners based on amides of L-aspartyl-D-amino acid [156], as well as in the synthesis of the β-lactam antibiotic (+)-thienamycin [157].

Racemic 1-phenylethanolamine can be produced the reductive amination of acetophenone in the presence of ammonia, using several catalytic approaches. A Raney nickel catalyst has been used in a methanol solution containing ammonia [158, 159] or an ethanol solution saturated with ammonia [160]. Other catalysts include platinum oxide in a methanol solution saturated with ammonia, containing an excess of ammonium chloride [161], and cobalt carbonyl with tributylphosphine in an ethanol solution. Other synthetic routes to 1-phenylethanolamine include deoxygenative ammination in a modified version of the Gabriel amine synthesis using tosylhydrazones as alkylating agents [162] and the N-alkylation of ammonia with 1-phenylethanol catalyzed by Ni/CaSiO3 nanoparticles [163].

The enantiomers of 1-phenylethanolamine can be obtained by various asymmetric syntheses. (S)-(−)-1-Phenylethanolamine is produced in the Lossen rearrangement of (+)-N-hydroxy-2-phenylpropanamide as well as the Schmidt rearrangement of (+)-2-phenylpropanoic acid [164], or the Hofmann reaction of (+)-2-phenylpropanamide [165]. (R)-(+)1-Phenylethanolamine can be produced by the spiroborate-catalyzed reduction of (Z)-1-phenylethanolone oxime [166]. Either enantiomer of 1-phenylethanolamine can be produced in enantiopure form and approximately 60% yield by the
enzymatic transformation of acetophenone using ω-transaminases. [167].

The chiral resolution of 1-phenylethanamine has been reported by diastereomer crystallization with malic acid (mutual resolution, as mentioned above [153]), tartaric acid [168] or cinnamic acid [169]. According to the Marckwald principle, either enantiomer can be crystallized depending on the configuration of the acid used as resolving agent. The enzymatic kinetic resolution of 1-phenylethanamine has been realized by the selective conversion of (S)-(−)-1-phenylethanamine to acetophenone using an *Ochrobactrum anthropi* ω-transaminase [170], as well as the enantioselective acylation of (R)-(+)−1-phenylethanamine using immobilized *Candida antarctica* lipase B [171, 172]. Dynamic kinetic resolutions of 1-phenylethanamine has been performed with the selective acylation of the (R) enantiomer with *Candida antarctica* lipase B (Novozym 435) and racemization over a Pd catalyst [173, 174].

1.5.2. (S)-(−)-2-(N-Benzylamino)butan-1-ol

The S configuration of 2-(N-benzylamino)butan-1-ol, used for the resolution of cPA, is shown in Figure 14.

![Structure and configuration of (S)-(−)-2-(N-benzylamino)butan-1-ol](image)

Figure 14: Structure and configuration of (S)-(−)-2-(N-benzylamino)butan-1-ol.

Initially, 2-(N-benzylamino)butan-1-ol attracted interest as the intermediate in the synthesis of local anaesthetics [175, 176], however, these reports do not make the distinction between the two enantiomers. Other applications of 2-(N-benzylamino)butan-1-ol as a chiral building block or as a resolving agent typically utilize the R enantiomer. However, as mentioned above, the resolution of cis-permethric acid via diastereomer crystallization was reported using (S)-(−)-2-(N-benzylamino)butan-1-ol as the resolving agent [142, 144]. Additionally, the synthesis of a phosphoinositide-dependent protein kinase-1 (PDK1) inhibitor (a potential anticancer agent) has been realized using (S)-(−)-2-(N-benzylamino)butan-1-ol as a chiral intermediate [177].
Synthetic routes to 2-(N-benzylamino)butan-1-ol typically employ 2-aminobutanol as their starting material, which is itself a chiral molecule. Since this reagent is readily available in both racemic and enantiopure forms, often the main difference between racemate and asymmetric syntheses is the configuration of the starting aminobutanol.

The first reported synthesis of racemic 2-(N-benzylamino)butan-1-ol has been described by Pierce et al. [175], via the in situ reaction between 2-aminobutanol and benzyl chloride by heating at 100 °C under reflux. Other non-enantioselective routes typically involve the reductive amination of benzaldehyde with 2-aminobutanol. This can be accomplished via catalytic hydrogenation using palladium on activated carbon [176, 178] or using sodium cyanohydridoborate (NaBH\(_3\)CN) [179].

Reductive amination of benzaldehyde has been performed by reacting it with the enantiopure 2-aminobutanol and treating the mixture with an ethanol solution of sodium borohydride (NaBH\(_4\)) [177, 180]. (S)-(+-)-2-(N-Benzylamino)butan-1-ol has also been obtained by the N-alkylation of benzylamine in a biomimetic electrocatalytic process [181].
2. MATERIALS AND METHODS

2.1. MATERIALS USED

Racemic ibuprofen (> 98.0%, GC) was purchased from TCI Europe N.V. (Zwijndrecht, Belgium). Racemic cis-permethric acid was synthesized in-house (> 98%, GC). (R)-(+-)-1-Phenylethanamine (≥ 99%, GC) was purchased from Merck Hungary Ltd. (Budapest, Hungary). (S)-(+-)-2-(N-Benzylamino)butan-1-ol was synthesized in-house (97.1%, GC) [182]. Perfil P250 was kindly given by Baumit Ltd. (Budapest, Hungary).

Carbon dioxide (99.5%) was purchased from Linde Gas Hungary Co. (Répcelak, Hungary). Methanol and ethanol (> 99.9%, GC) were purchased from Merck Hungary Ltd. (Budapest, Hungary).

2.2. VIEW CELL MEASUREMENT METHODS

2.2.1. EQUIPMENT

The variable-volume optical cell was manufactured by New Ways of Analytics (Lörrach, Germany). The schematic is shown in Figure 15.

The view cell (1) is equipped with a magnetically coupled stirrer (2) and a sapphire viewing window on a removable front wall. The temperature inside the cell is measured by a thermoelement connected to a temperature sensor (3), and controlled by electrical heating rods in the wall of the cell connected to a temperature controller (4). The pressure inside the cell is measured by a pressure transducer (5). The cell volume can be varied between approximately 40 and 70 ml by moving the rear wall by means of compressed air (6) fed to a hydraulic control cylinder (not pictured). The air pressure is controlled via a manual valve (not pictured) and the current cell volume is measured by a calibrated position sensor (7). The measured pressure, temperature and volume data is sent through an ADC (not pictured) and recorded by a computer (8). Carbon dioxide is fed into the reactor by a piston pump (9) through an inlet valve (10). The contents of the cell can be released through two outlet valves (11, 12), either for depressurization or for sampling the contents of the cell. The upper (11) and lower (12) valves are connected to ports near the top and bottom of the cell, respectively, thus different regions of the cell can be sampled independently (e.g. a liquid phase at the bottom can be sampled through the bottom valve, while the gas
Figure 15: Schematic of the high-pressure view cell. 1: high-pressure cell, 2: stirrer, 3: temperature sensor, 4: temperature controller, 5: pressure transducer, 6: compressed air, 7: position sensor, 8: computer, 9: piston pump, 10: inlet valve, 11: upper outlet valve, 12: lower outlet valve.

The phase can be sampled through the top valve. For clarity, the proportions have been exaggerated, in reality the inlet and outlet valves are connected to the cell by short sections of tubing, in order to minimize dead volume.

For the applications described in the following sections, the mass of CO$_2$ delivered by the piston pump must be known. The pump computes the available cylinder volume from the piston position at a resolution of 0.01 ml. Recording the changes in this volume can be used to compute the mass of CO$_2$ if its density inside the cylinder is known. To this end, the temperature and pressure of CO$_2$ must be controlled and monitored. The temperature of the cylinder – chosen such that the CO$_2$ inside it is liquid and therefore less compressible – is controlled by tempering with water circulating in the jacket, the temperature of which is monitored. The piston pump is programmed to maintain a constant pressure inside the cylinder. Therefore, the temperature and pressure of CO$_2$ is known, thus its density can be calculated and – since the movement of the piston is slow enough to minimize compressibility – assumed to
be practically constant.

The maximum operating pressure and temperature of the view cell is 750 bar and 150 °C. Solid materials can be loaded into the cell by removing the front wall. In order to measure solutions into the cell, the thermoelement is first removed from the assembled cell and the solution is pipetted in through the empty socket.

2.2.2. Solubility Measurement Method

As detailed in Section 2.3.3, the in situ resolution method requires that the complete dissolution of certain compounds in a known quantity of carbon dioxide be ensured. To this end, the solubility of these compounds must be determined. Although the solubility can be estimated from extraction curves [108], the method described below is more accurate and can be used to measure solubility under different parameters without any intervening loss of material.

The solubility of a given compound can be characterized by the saturation concentration $x$. In the case of (supercritical) carbon dioxide, the solvent power depends on the density $\rho$ and the temperature $T$. Additionally, density itself is a function of pressure and temperature: $\rho(p, T)$. Therefore, solubility in CO$_2$ or scCO$_2$ is described by the triad of values $(x, p, T)$. The variable volume and controlled temperature of the view cell allows the pressure to be varied while keeping $T$ and $x$ constant, and thus lends itself naturally to the measurement of oversaturation pressures $p(T, x)$ (also known as cloud points).

First, the cell is brought to its minimum volume, tempered at the first desired temperature ($T_1$), and a known mass of material is measured into it. The cell is filled with CO$_2$ from the piston pump until the material is completely dissolved. The mass of carbon dioxide is calculated by the method described in Section 2.2.1. From this mass, as well as the mass of material in the cell, the (mass or mole) fraction of the dissolved material ($x_1$) is calculated. The cell volume is slowly increased until phase separation appears, the pressure at this point is recorded as the cloud point (oversaturation point) $p_o(T_1, x_1)$. The cell volume is then slowly decreased until complete redissolution, the pressure at which this occurs is the dissolution point $p_d(T_1, x_1)$. By tempering the cell to a different temperature and repeating the above procedure, the cloud points $p_o(T_2, x_1)$, $p_o(T_3, x_1)$ etc. and the corresponding dissolution points can be measured. By filling additional CO$_2$ into the cell (the mass of which can be determined using the method outlined above), cloud points at the known composition $x_2 < x_1$ can be determined. Combined with measurements at different temperatures ($T_1$, $T_2$ etc.),
the saturation properties \( p_o(T_n, x_n) \) and \( p_d(T_n, x_n) \) (where \( n \) is typically 3) can be measured.

### 2.2.3. Antisolvent Screening Method

The aim of this method is to identify a range of process parameters in which the antisolvent crystallizations described in Sections 2.3.4 and 2.4.2 can proceed. As detailed in the later sections, these crystallizations involve contacting materials dissolved in an organic solvent with (supercritical) carbon dioxide. The process parameters of primary interest (besides pressure and temperature) are the concentration of (one or more) compounds in the organic solvent \( (m_{\text{material}}/V_{\text{solvent}}) \), the "volumetric" concentration of (one or more) compounds (e.g. the ratio of their mass to the volume of the containing vessel, \( m_{\text{material}}/V_{\text{vessel}} \)) and the mass ratio of organic solvent to carbon dioxide \( (m_{\text{solvent}}/m_{\text{CO}_2}) \).

The cell is assembled, tempered, set to a desired volume \( (V_{\text{vessel}}) \), and a concentrated (near-saturation) solution (of known \( m_{\text{material}} \) and \( V_{\text{solvent}} \)) of the compound(s) of interest is measured into it. Carbon dioxide is filled into the cell from the piston pump, the mass of \( \text{CO}_2 \) \( (m_{\text{CO}_2}) \) can be calculated by the steps described in Section 2.2.1. The contents of the cell are observed visually and the behaviour of the system is noted. If precipitation appears, the solution can be stirred to ensure that the solid phase does not redissolve (as is the case with temporary precipitation that appears due to local oversaturation, or because the \( \text{CO}_2 \), being delivered from the low-temperature piston, cools the cell contents to below the solubility limit). The \( \text{CO}_2 \) phase can be sampled by releasing a small amount of it through the upper outlet valve (so as to disturb the solid phase as little as possible). In order to study the precipitate, the cell must be depressurized, however, if this is done while the organic solvent is present in the \( \text{CO}_2 \) phase, depressurization will cause it to condense in liquid form, potentially dissolving part or all of the solid phase. To avoid this, additional carbon dioxide at a constant pressure is introduced into the cell from the piston pump while opening the upper outlet valve. By carefully controlling the outlet valve, the flow rate can be kept at a low value so that the amount of solid material carried out of the cell by the \( \text{CO}_2 \) stream is minimal, in turn minimizing precipitate losses and the risk of blockage.
2.3. **Batch Resolution Methods**

2.3.1. **Equipment**

The high-pressure reactor used for the batch experiments was manufactured by the Research Institute of Applied Chemistry at the University of Miskolc. The reactor can be used at pressures up to 250 bar and has an internal volume of approximately 36 ml (see below).

Figure 16 shows a schematic of the reactor assembly. A piston pump (1) delivers CO\(_2\) through an inlet valve (2) to the reactor vessel (3). Conditions inside the vessel are monitored by a pressure transducer (4) and a thermocouple (5), connected to a computer (not pictured) recording their data. The vessel is tempered by water (6a, 6b) circulated in the jacket of the vessel by a thermostat (not pictured). Stirring is accomplished by an external magnetic stirrer (7) and a stir bar inside the reactor (8). Carbon dioxide leaving the reactor passes through a filter (9) and an outlet valve (10), causing an expansion to essentially atmospheric pressure. The expansion causes any compounds dissolved in the CO\(_2\) to precipitate, these are collected in a liquid trap (11).

The internal volume of the reactor varies slightly due to the differing volumes of fittings, stir bars, etc. Should an exact value be required, it can be obtained by

![Figure 16: Schematic of the batch reactor system. 1: piston pump, 2: inlet valve, 3: reactor vessel, 4: pressure transducer, 5: thermocouple, 6: tempering water, 7: magnetic stirrer, 8: stir bar, 9: filter, 10: outlet valve, 11: liquid trap.](image-url)
calibration, making use of the fact that since the reactor uses the same piston pump as the view cell, the mass of CO$_2$ delivered into the vessel can be calculated by the method described in Section 2.2.1. The reactor is tempered and pressurized with CO$_2$ and allowed to come to thermal equilibrium in order to reduce density variations inside the vessel. The density of CO$_2$ is calculated from the measured pressure and temperature inside the reactor, the mass of CO$_2$ is determined from data measured by the piston pump (see Section 2.2.1). From the known mass and density, the volume of CO$_2$ can be calculated. By changing the temperature of the tempering water and allowing thermal equilibrium to re-establish, the density and hence the volume can be calculated from different pressure–temperature pairs, reducing the effect of measurement errors.

The maximum operating temperature of the batch reactor – due to water being used as a tempering medium – is 95 °C, the maximum operating pressure is 225 bar. Similar to the operation of the view cell, filling the reactor is done by operating the piston pump at a constant pressure in excess of the desired reactor pressure. This ensures that a pressure differential always exists across the inlet valve and that CO$_2$ always flows towards the reactor, preventing contamination of the CO$_2$ supply system.

The CO$_2$ phase inside the reactor can be sampled by setting the piston pump to operate at a constant pressure, however, in contrast to filling, it is set to maintain the same pressure as that in the reactor. Sampling is started by opening the outlet valve briefly, allowing the pressure inside the reactor to drop slightly (usually 3–5 bar, cf. typical reactor pressures of 100–200 bar), ensuring that the reactor pressure is below that maintained by the piston pump. The inlet valve is opened, and by careful adjustment of the outlet valve, the piston pump will maintain a constant pressure across the system (up to the outlet valve) while CO$_2$ flows through the reactor and into the liquid trap. As seen in Figure 16, CO$_2$ is delivered to the bottom of the reactor by means of tubing attached to the inlet fitting, thus a large fraction of the reactor's internal volume is affected, leading to representative sampling of the CO$_2$ phase. The piston pump is used to track the volume of CO$_2$ delivered to the reactor, typically 1–5 ml per sample. Sampling is ended by shutting off the inlet valve, immediately followed by closing the outlet valve. The outlet valve is washed – typically with the same solvent as is used in the liquid trap – to collect any materials that precipitated and were deposited inside it.

Separating the compounds in the CO$_2$ phase from precipitated solids is accomplished by washing out the reactor. The procedure for this is almost identical to sampling technique described above, with two key differences. First, the amount of CO$_2$
is typically 2–3 times that of the reactor, in the range of 60–100 ml, in order to remove as much of the dissolved compounds as possible (see Section 2.6.2). Second, washing is ended by only shutting off the inlet valve, allowing the reactor to depressurize. After depressurization and disassembly, precipitates can be removed from the reactor in solid form (or, in the case of minuscule amounts, dissolved in organic solvents and pipetted out).

Since the separation step can be considered an extraction, nomenclature is used accordingly: any materials remaining in the reactor after washing are referred to as the raffinate, while the CO\textsubscript{2}-soluble compounds recovered from the liquid trap are referred to as the extract.

### 2.3.2. **In vacuo METHOD**

This resolution technique is based on earlier experiments by our group, using packed column extraction. The diastereomer formation actually occurs before the materials are loaded into the reactor. However, in contrast to packed column extraction, in which materials are contacted with scCO\textsubscript{2} for short periods of time (on the order of tens of minutes), this technique relies on prolonged exposure of the diastereomers and the unreacted enantiomers to scCO\textsubscript{2}, and the changes that said exposure induces in the structure, optical purity etc. of the materials.

The racemate and the resolving agent are dissolved in an organic solvent, which is then evaporated under vacuum. If the evaporation product is not suitable for handling (e.g. too small in quantity, does not solidify), the inert support Perfil P250 (expanded and milled perlite used as a filtration aid, specific surface area 2.89 m\textsuperscript{2}/g) is added to the solution. The solid remaining after evaporation is loaded into the tempered reactor, which is then sealed and filled with CO\textsubscript{2} to the desired reaction pressure, at which point the stirring is activated and the reaction is assumed to start.

Samples are taken from the CO\textsubscript{2} phase during the experiment according to the steps presented in Section 2.3.1. After the desired reaction time elapsed, the reactor is washed and depressurized, and the products are collected, also by the method described in Section 2.3.1.

### 2.3.3. **In situ METHOD**

Although CO\textsubscript{2} is a widely used reaction medium, it is typically utilized for homogeneous-phase reactions in scCO\textsubscript{2} or heterogeneous-phase reactions between liquid
CO$_2$ and liquid reagents. The *in situ* technique, on the other hand, involves the heterogeneous-phase reaction of solid and liquid reagents in scCO$_2$, and therefore represents a novel method for the production of enantiomers. Additionally, scCO$_2$ is used as an extraction medium as well, for the separation of the unreacted enantiomers from the precipitated diastereomeric mixture.

The racemate and the resolving agent are loaded into the reactor separately without any solvent. Special precautions were required for experiments involving PhEA, because when exposed to air, PhEA reacts with atmospheric CO$_2$ to form a self-derivative carbamate compound [183]. Although, naturally, there is evidence that this carbamate formation also proceeds in scCO$_2$, when liquid PhEA is left exposed to air for extended periods of time, it tends to form a hard layer along the surface of the container. This could influence reaction kinetics, or, in extreme cases, immobilize the stir bar. Therefore, care was taken to ensure that the reactor could be sealed and filled with CO$_2$ as soon as possible after pipetting liquid PhEA into it. The reactor vessel is sealed and pressurized with CO$_2$ to the desired reaction pressure, at which point stirring is activated and the reaction is assumed to start. Sampling, washing and depressurization of the reactor proceeds by the methods described in 2.3.1.

The main advantage of this method over the *in vacuo* technique is that it completely forgoes the use of organic solvents. The reaction and separation steps both use only supercritical carbon dioxide as a solvent. Although there is organic solvent used for the liquid trap, this is only a consequence of the experiments being carried out at the laboratory scale. In a pilot or industrial scale plant, the liquid trap would be most likely replaced with a bag filter, cyclone or other suitable solids collection device.

### 2.3.4. **Gas Antisolvent (GAS) Method**

Despite what the nomenclature seems to suggest, this technique uses scCO$_2$ as an antisolvent. In the literature of antisolvent processes, batch methods are referred to as gas antisolvent (GAS) techniques, while semi-continuous or continuous methods are referred to as supercritical antisolvent (SAS) techniques (see Section 2.4).

In this method, a concentrated (near-saturation) organic solution of the racemate and resolving agent is prepared and measured into the tempered reactor. The vessel is sealed and pressurized with CO$_2$ to the desired reaction pressure. The mixture is stirred to ensure thorough mixing of the organic solvent with CO$_2$ and, therefore, to ensure that equilibrium is established and that kinetic effects are minimized. Sam-
pling, washing and depressurizing the reactor can be carried out according to the procedure detailed in 2.3.1.

In addition to the factors that characterize the in vacuo and in situ resolutions (reactor pressure and temperature, reaction time, racemate amount, molar ratio), GAS experiments are also affected by the ratio of the solvent and the antisolvent. Denoted by $R$, it was defined based on the masses ($m$) of carbon dioxide and the organic solvent (indices $CO_2$ and solvent, respectively):

$$R = \frac{m_{CO_2}}{m_{solvent}}$$ (2.1)

Since $R$ is technically a dimensionless number, its values are given without units, in the form of ratios (e.g. "3:1"). However, for the sake of clarity, its units may be specified as either [-] (dimensionless) or the equivalent [g/g].

For comparing experiments carried out with different solvents (having different molar masses), the ratio may be defined in terms of molar quantities ($n$):

$$R_m = \frac{n_{CO_2}}{n_{solvent}}$$ (2.2)

Similar to $R$, values of $R_m$ are given as ratios (e.g. "9.3:1") and its units may be specified as [-] (dimensionless) or as [mol/mol].

A significant advantage of this method is that it reduces the complexity of material preparation steps prior to loading the reactor. This results in significantly shorter operating times when compared to the in situ method (however, the in situ method retains the advantage of completely eliminating the use of organic solvents). Operating times are also shorter compared to the in vacuo technique, with roughly comparable use of organic solvent.

### 2.4. Supercritical Antisolvent (SAS) Method

#### 2.4.1. Results of Apparatus Development

The semi-continuous crystallizer is based on the laboratory-scale supercritical extractor used by our group for earlier experiments. The extractor has been replaced with a crystallization chamber and equipped with an automated solution injection system. These upgrades, including the development of the automated injection system (as detailed below), were the results of my doctorate studies.

A simplified schematic of the apparatus is shown in Figure 17. Carbon dioxide

is supplied from a commercial gas cylinder (1), inverted to ensure that liquid CO₂ is being drawn (alternatively, dip-tube cylinders could be used). Carbon dioxide from the cylinder enters a cooler (2) where its temperature drops to approximately 5 °C, in order to minimize its vapor content before entering the pump (3), where it is compressed above its critical pressure. After being heated above its critical temperature in a heat exchanger (4), it enters the crystallization vessel (5). Conditions inside the vessel are monitored by a pressure transducer (6) and a thermocouple (7). The vessel is tempered by a water bath (omitted for clarity). Organic solutions are delivered by an HPLC pump (H1) drawing from a solution container (C1), through a 6-port switching valve (V). For the detailed operation of the automated injection system that includes parts H1, C1 and V, refer to the description of Figure 18. The solution inlet line is
equipped with a check valve (8) to prevent CO$_2$ from entering the 6-port valve, as the small internal volumes of that part make it especially susceptible to blockage from unintended precipitation. CO$_2$ exiting the crystallizer vessel passes through a filter (9) to minimize precipitate losses, then through an expansion valve (10), where its pressure drops to below critical. Dissolved compounds that precipitate due to the pressure drop are collected in the separator vessel (11). Carbon dioxide exiting the vessel is expanded to atmospheric pressure in an outlet valve (12) and two expansion vessels (not pictured). The mass flow rate of carbon dioxide is measured by an electronic mass flow meter (13) and its cumulative volume is measured by a conventional gas meter (14). CO$_2$ exiting the gas meter is exhausted into the atmosphere. The maximum operating temperature is limited to 95 °C (due to water being used as the tempering medium), the maximum operating pressure is 200 bar.

Figure 18 shows the schematic of the automated solution delivery system. The hardware connections and software were developed in-house as part of my studies. Parts H1, C1 and V correspond to the respectively labelled parts on Figure 17: a Gilson 305 HPLC pump (H1) draws from a solution container (C1) and pumps it towards a manually operated six-port switching valve (shown in its two positions as V_a and V_b). Ports on the valve from which no lines are drawn extending outwards are sealed, thus in position V_a, the switching valve isolates the HPLC outlet line from the crystallizer (represented by the line downwards, cf. Figure 17). In this position, any liquid exiting pump H1 is routed towards a waste container (represented by the arrow pointing left). Therefore, position V_a is used for rinsing the system or priming pump H1. Actuating the valve to position V_b connects the HPLC outlet line to the crystallizer (through the check valve, see part 8 on Figure 17). This is done manually, and only after pump H1 is started, as an added precaution to the check valve. For the same reason, the valve is actuated back to position V_a before pump H1 is stopped.

Pure solvent can be delivered into the solution container C1 by a Gilson 303 HPLC pump (H2), which draws from a solvent reservoir (C2). Solvent is transferred from C2 to C1 either to provide pure solvent for rinsing pump H1, or during a measurement in order to prevent pump H1 from drawing air due to the fluid level in the container C1 decreasing below the inlet line.

This latter condition is avoided by gravimetric monitoring: the container is situated on an electronic scale (SC) equipped with a standard RS-232 terminal, the data from which is relayed through an RS-232–USB adapter (A1) to a computer (PC) which processes and logs the weight readings. The computer controls the two HPLC pumps and responds to user input via a two-port RS-422–USB adapter (A2).

User input consists of a start button (ST) and an emergency shutdown button (SH). These are momentary, push-to-make buttons that connect the CTS (Clear to Send) and GND (Ground) pins of each port on the adapter, interpreted by the hardware as setting the CTS line.

Control of the HPLC pumps is accomplished through the RTS (Ready to Send) and TXD (Transmit) pins of the RS-422 adapter, both of which can be set to maintain either high or low voltage levels (the latter by setting or clearing the Break line). The signal levels of the RS-422 standard (0 V and +5 V) make it directly compatible with the TTL inputs of the Gilson 305, allowing the computer to start and stop the pump, as well as to change the flow rate. This latter function is accomplished by predefined pump programs, stored by the Gilson 305. Each pump program sets the flowrate to
a user-specified value, then waits for a TTL signal (triggered by the software when a change in flow rate is desired), upon reception of which the pump program finishes. Each pump program can be optionally instructed to execute one other pump program when it finishes. Thus, several programs may be chained together to form a sequence of flow rates, through which the software can advance as desired.

The Gilson 303 can be triggered to dispense a fixed amount of solvent at a predefined flowrate by shorting a pair of contacts, this is accomplished by a reed relay (not pictured) in order to maintain electrical insulation. Additionally, a manual refill button (RF) can be used to trigger the Gilson 303 at the user's discretion.

The software was written in Visual Basic for Applications, using an open-source library to handle serial communication. Due to hardware limitations, interrupts could not be used, thus serial ports are polled at intervals of 1 ms. Weight readings are timestamped with the same 1 ms resolution. In addition to the graphical user interface, audible warnings are provided for certain events (such as starting or stopping the Gilson 305, so that the six-port valve can be actuated).

Measurement programs are user-editable, consisting of predefined commands (such as starting or stopping the Gilson 305, triggering the Gilson 303, starting or stopping data logging, etc.) which are executed in sequential fashion (no conditional branches or loops are supported). After a command is completed, command processing is paused for the length of a user-defined delay, then resumes with the next command in sequence. With some exceptions, commands are completed as soon as their code is executed. The exceptions are special commands which wait for certain events to occur: the user pressing the start button, the digital scale reporting its weight reading as stable (to ensure, for example, that the Gilson 303 is finished delivering solvent into the solution container), or special user-defined conditions (called triggers) being met.

Triggers halt program execution until the weight reading has decreased below or increased above a certain target value. The target value can be absolute (compared to the weight reading without modification), relative to a previously stored value, or percentages computed from two previously stored values (corresponding to 0% and 100%). Triggers are used, for example, to ensure that the container C1 does not run out of solvent, by signaling the Gilson 303 (H2) to deliver solvent from container C2 to C1 when the weight reading decreases below a certain limit.
2.4.2. Measurement Technique

As stated in Section 2.3.4, SAS denotes precipitation methods using supercritical antisolvent which proceed continuously or semi-continuously.

The method involves preparing a solution of the racemate and the resolving agent, and measuring it into the solution container. The software records the tare weight of the container, so that the net weight of the solution can be monitored accurately. The flow of CO$_2$ is started, and pressure is set to the desired values in the crystallizer and separator (100–200 bar and 40 bar, respectively) by adjusting the expansion and outlet valves. Once the pressures have stabilized, injection of the solution is initiated by pressing the start button. When the solution weight decreases below 10% of its initial value, the software triggers pump H2 to deliver solvent into the solution container. Once only 20% of this diluted solution remains, pumping is halted by the software. Once the six-port valve is actuated so that HPLC system is isolated from the crystallizer, the software can be signalled to clean the HPLC tubing with pure solvent. In order to ensure that no organic solvent remains in the crystallizer (which could possibly redissolve precipitates during depressurization), it is washed with additional scCO$_2$. The flow of CO$_2$ is then stopped, the equipment is depressurized and the products are collected. Precipitates in the crystallizer (referred to as the raffinate) are recovered in solid form, while CO$_2$-soluble materials (referred to as the extract) are typically recovered from the separator along with (and dissolved in) the organic solvent from the initial solution.

Although the semi-continuous nature of this technique is materially different from that of the GAS method, a ratio between the solvent and antisolvent can be defined analogously to $R$ (see Section 2.3.4, Eq. 2.1 on p. 38). When both streams are being pumped into the crystallizer vessel, and steady state has been attained, the mass flows ($\dot{m}$) of carbon dioxide and the organic solvent (indices CO$_2$ and solvent, respectively) can be used to calculate the mass flow ratio:

$$R = \frac{\dot{m}_{CO_2}}{\dot{m}_{solvent}}$$ (2.3)

As with $R$ defined in Eq. 2.1, values are given without units, as ratios (e.g. "2.4:1"). Units may be specified either as [-] (dimensionless) or as the equivalent [g/g].

During the preparation of the starting solution, special steps are taken so that the mass composition of the solution can be estimated. The tare weight of an empty container is measured and the racemate and resolving agent are added into it. The
weight of these latter two are determined independent of the container weight. The organic solvent is added, the container is sealed to prevent solvent loss due to evaporation, and the total weight of the container is measured. By subtracting the previously recorded tare weight, the weight of the solution in the container is obtained. Since the solution consists only of the racemate, the resolving agent and the solvent, and the weights of the former two are known, the mass fractions of all components can be calculated. The solution is then transferred to the injection container (labelled C1 on Figs. 17 and 18), the tare weight of which is recorded by the control software. Thus, the weight of the solution in the injection container C1 is known, and any losses are considered not to affect the mass composition. From the solution weight and the mass fractions, the weight of the racemate and the resolving agent in the injection container can be computed. The control software estimates the injected amount of these components based on the weight changes reported by the scale. Control experiments suggest that this estimate is in good agreement with the amount of material recovered from the crystallizer vessel.

2.5. ANALYTICAL METHODS

2.5.1. CHIRAL GAS CHROMATOGRAPHY (GC)

This technique was used to determine the relative amounts of enantiomers in a sample. The peak areas obtained from chromatograms can be substituted directly into the equation for calculating ee values, see Section 2.6.1.

Measurements were carried out on a Finnigan FOCUS GC (Thermo Fischer Scientific, Waltham, MA, USA) fitted with a Supelco® BetaDEX™ 120 column (20% permethylated β-cyclodextrin, length 30 m, ID 0.25 mm, film thickness 0.25 µm). Samples were prepared in concentrations of 1–4 mg/ml, 0.6 µl was delivered manually into a split injector at 250 °C. Helium was used as carrier gas, detection was done by a flame ionization detector (FID) at 250 °C.

Parameters for ibuprofen: carrier pressure 140 kPa, split flow 50 ml/min, oven temperature 100 °C (2 min), ramp 20 °C/min, 160 °C (8 min), ramp 10 °C/min, 210 °C (8 min), ramp 10 °C/min, 230 °C (4 min).

Parameters for cis-permethric acid: carrier pressure 170 kPa, split flow 90 ml/min, oven temperature 110 °C (1 min), ramp 10 °C/min, 200 °C (3 min), ramp 10 °C/min, 230 °C (4 min).
2.5.2. Powder X-ray diffraction (XRD)

This technique was used for characterizing crystallization products. XRD is capable of identifying different crystalline phases, even if they are present as physical mixtures. In this latter case, the diffractogram is the superimposition of each constituent solid phase. Therefore, diffractograms of all pure compounds (racemic and enantiopure forms of the investigated racemates as well as the resolving agents employed) and of the diastereomers (prepared by evaporation as with the in vacuo technique, see Section 2.3.2) were recorded for reference and compared to diffractograms of the crystallization products. If the peak positions and relative intensities of the product diffractograms do not match those of the reference diffractograms, the product crystalline structure is proven to be distinct from those of the reference materials. The appearance of a new crystalline structure could indicate that the reference materials have crystallized with a different structure, or the formation of new chemical species, such as reaction byproducts. The product diffractograms can be compared against those of the diastereomer standards, to check whether the crystalline structures of salts formed under supercritical conditions are different from those prepared under vacuum. Trace contaminants cannot be detected using this method, but the relative peak areas/heights of different compounds in a sample do provide qualitative information about the relative quantities of said compounds.

Diffractograms were recorded at Budapest University of Technology and Economics, Department of Inorganic and Analytical Chemistry, using an X'Pert Pro MPD diffractometer (PANalytical, Almelo, The Netherlands) equipped with an X'celerator detector in Θ–Θ arrangement (moving source, moving detector). A copper tube was used as the beam source, applying 40 kV voltage and 30 mA current. Samples were irradiated at the Kα line of copper (1.5408 Å), the Kβ radiation was filtered out by a nickel foil. Diffractograms were typically recorded between 2Θ values of 4° and 42° with a 1 minute of angle (0.0167°) step size and 31.115 s counting time.

2.5.3. Scanning electron microscopy (SEM)

Certain peculiar characteristics of diastereomers formed by antisolvent crystallizations (see Section 3.3.1, p. 81) prompted a visual investigation of the crystals. Conventional microscopy was attempted but did not reveal sufficient detail, thus SEM was chosen to conduct a visual survey of the crystalline products.

Measurements were carried out at the Department of Inorganic and Analytical
Chemistry of the Budapest University of Technology and Economics, using a JEOL JSM 5500-LV (JEOL Ltd., Tokyo, Japan) electron microscope. Prior to the analysis, samples were coated with a 5–10 nm Au/Pd layer for conductivity. Images were captured using 20 kV voltage and a secondary electron detector.

2.6. Calculations

2.6.1. Optical purity

Optical purities were determined from gas chromatography analyses by substituting the peak areas ($A$) of the major and minor components (indices maj and min, respectively) into Eq. 1.1 (see p. 9):

$$\text{ee} = \frac{A_{\text{maj}} - A_{\text{min}}}{A_{\text{maj}} + A_{\text{min}}}$$

(2.4)

As stated in Section 1.1.2, ee values calculated from Eq. 1.1 (and thus, the analogous Eq. 2.4) are unambiguous only when the major component is explicitly specified along with the numeric value. However, certain situations, such as showing ee values with differing major components on the same graph, require a more compact notation. In these cases, special definitions of ee with fixed major components are used.

For ibuprofen, the special definitions $\text{ee}_{(R)}$ and $\text{ee}_{(S)}$ are used, indicating ee values with $\text{(R)}$-IBU and $\text{(S)}$-IBU, respectively, as the major component. For cis-permethric acid, the special definitions $\text{ee}_{(+)}$ and $\text{ee}_{(-)}$ are used, indicating ee values with $\text{(+)}$-cPA and $\text{(-)}$-cPA, respectively, as the major component.

The overall ee value of multiple chemically homogeneous fractions can be calculated as the mass-weighted average of their ee values:

$$\overline{\text{ee}} = \frac{\sum m_i \cdot \text{ee}_i}{\sum m_i}$$

(2.5)

In the above equation, $m$ represents mass and the index $i$ is a running variable denoting the individual fractions.

Strictly speaking, raffinate calculations should use diastereomeric excess instead of ee. However, if the separation of the unreacted enantiomers from the diastereomeric salts is not perfect, the raffinate will contain both diastereomers characterized by de and enantiomers characterized by ee. The diastereomers, however, are decomposed in the inlet of the gas chromatograph, and the resulting enantiomers are mixed.
with the unreacted enantiomers left behind by the imperfect separation. Therefore, the GC reports an overall ee value for the entire fraction, which is – effectively – a weighted average of the ee of the enantiomers formed by the decomposition of the salts and the ee of the unreacted enantiomers. This overall ee value is used to characterize raffinate optical purities.

2.6.2. Yield

In the most general approach, the yield \((Y)\) of the extract and raffinate can be calculated by comparing them to the total amount of starting material:

\[
Y_e = \frac{m_e}{m_rac + m_{res}} \tag{2.6a}
\]

\[
Y_r = \frac{m_r}{m_rac + m_{res}} \tag{2.6b}
\]

In the definitions above, \(m\) denotes mass, the indices \(e\) and \(r\) refer to the extract and raffinate and the indices \(rac\) and \(res\) refer to racemate and resolving agent. Unlike the approaches presented below, this definition can be used regardless of the experimental technique. However, a significant disadvantage of this approach is that yields are not normalized, thus for different racemate–resolving agent pairs, the yield values will vary depending on the molar masses and molar ratios (mr) of the compounds involved.

One approach for normalizing yields is to use an ideal resolution as the reference, assuming a complete, irreversible equimolar reaction between the resolving agent and the racemate:

\[
\hat{Y}_e = \frac{m_e}{(1 - mr) \cdot m_{rac}} \tag{2.7a}
\]

\[
\hat{Y}_r = \frac{m_r}{mr \cdot m_{rac} + m_{res}} \tag{2.7b}
\]

As with Eq. 2.6a and 2.6b, \(m\) denotes mass, the indices \(e\) and \(r\) refer to the extract and raffinate and the indices \(rac\) and \(res\) refer to racemate and resolving agent, while \(mr\) refers to the molar ratio. The multiplication of the molar ratio (defined in Eq. 47, 48).
1.2 as the ratio of two molar quantities) by a mass, although unusual, does in fact produce correct dimensions. Consider the product $mr \cdot m_{\text{rac}}$ in the denominator of Eq. 2.7b and substitute the definition of $mr$ from Eq. 1.2:

$$mr \cdot m_{\text{rac}} = \frac{n_{\text{res}}}{n_{\text{rac}}} \cdot m_{\text{rac}}$$

Using the identity $n = \frac{m}{M}$, where $n$ denotes molar quantity, $m$ denotes mass and $M$ denotes molar mass, yields:

$$mr \cdot m_{\text{rac}} = \frac{n_{\text{res}}}{m_{\text{rac}}} \cdot M_{\text{rac}}$$

$$mr \cdot m_{\text{rac}} = n_{\text{res}} \cdot M_{\text{rac}}$$ \hspace{1cm} (2.8)

The physical interpretation of Eq. 2.8, considering the identity $n \cdot M = m$ (where, as above, $n$, $M$ and $m$ denote molar quantity, molar mass and mass, respectively), is a mass of the racemate equal in molar amount to the resolving agent, i.e. the mass of racemate consumed in an equimolar reaction with the resolving agent. A similar derivation starting from the denominator of Eq. 2.7a yields:

$$(1 - mr) \cdot m_{\text{rac}} = (n_{\text{rac}} - n_{\text{res}}) \cdot M_{\text{rac}}$$ \hspace{1cm} (2.9)

The physical interpretation of Eq. 2.9 is the mass of the racemate minus the mass equal in molar amount to the resolving agent, i.e. the mass of unreacted racemate left over after an equimolar reaction with the resolving agent.

In principle, values of $\hat{Y}$ range between 0–1, although both $\hat{Y}_e$ and $\hat{Y}_r$ can exceed 1 under certain conditions. If the extraction of the unreacted enantiomers is incomplete (due to factors such as inadequate stirring or insufficient amount of scCO$_2$ used for the extraction), the increased amount of racemate enantiomers in the raffinate can cause $\hat{Y}_r$ to exceed 1. On the other hand, if the diastereomer formation does not proceed to completion or is not irreversible, the increased amount of unreacted enantiomers in the extract can cause $\hat{Y}_e$ to exceed 1.

Although the incorporation of $mr$ eliminates the variation of $\hat{Y}$ with the molar masses of the racemate and resolving agent, it introduces an issue: the values, as defined in Eq. 2.7, are only valid if $mr < 1$. If $mr = 1$, the value of $\hat{Y}_e$ is invalid (division by zero), while for $mr > 1$, $\hat{Y}_e$ is negative and the denominator of $\hat{Y}_r$ is
physically invalid as it exceeds the total mass of material used for the resolution (due
to the term \(mr \cdot m_{\text{rac}}\) where \(mr > 1\)). Although the latter issue could be rectified
by replacing \(mr\) in the denominator Eq. 2.7b with the expression \(\min(1; mr)\), Eq.
2.7a cannot be augmented to yield correct values for \(mr \geq 1\). If there is significant
dissociation of the diastereomeric salts (as seen, for example, in Section 3.1.2), the
resolution might be successful at or above \(mr = 1\), and therefore \(\tilde{Y}\) cannot be used
for these systems.

For experiments carried out in the batch reactor, the extraction efficiency can be
taken into consideration by treating the reactor as a continuously stirred tank reac-
tor (CSTR). For a dissolved compound, let \(\dot{m}(t)\) and \(m(t)\) refer, respectively, to its
effluent mass flow rate and dissolved mass in the reactor, at time \(t\). According to the
conservation of mass, the following are related by the following equation:

\[
\dot{m}(t) = -\frac{dm(t)}{dt}
\]

Substituting \(\dot{m}(t) = \dot{V}(t) \cdot c(t)\), where \(\dot{V}\) and \(c(t)\) denote the effluent volumetric
flow rate and the effluent concentration, respectively, at time \(t\), yields:

\[
\dot{V}(t) \cdot c(t) = -\frac{dm(t)}{dt}
\]

The shorthand notation \(\dot{V}(t)\) is expanded to \(\frac{dV(t)}{dt}\), where \(V(t)\) denotes the cumu-
lative effluent volume at time \(t\), and the resulting equation is simplified:

\[
\frac{dV(t)}{dt} \cdot c(t) = -\frac{dm(t)}{dt}
\]

The effluent concentration at time \(t\) is given by the mass of the dissolved com-
 Pound in the reactor \(m(t)\) and the reactor volume \(V_{\text{reactor}}\) by \(c(t) = \frac{m(t)}{V_{\text{reactor}}}\). Substituting
this into the equation above and separating the variables yields:

\[
\begin{align*}
\frac{dV(t)}{V_{\text{reactor}}} \cdot \frac{m(t)}{V_{\text{reactor}}} &= -\frac{dm(t)}{m(t)} \\
\frac{dV(t)}{V_{\text{reactor}}} &= -\frac{dm(t)}{m(t)}
\end{align*}
\]

(2.10)
Let $t = 0$ denote the start of the extraction, let $V(0) = 0$ and let $V_{\text{extraction}}$ denote the volume of CO$_2$ used for the extraction (at the pressure and temperature of the reactor). Furthermore, let $m_0$ and $m$ denote the mass of the material at the start and the end of extraction, respectively. Integrating Eq. 2.10 gives:

$$\frac{1}{V_{\text{reactor}}} \int_0^{V_{\text{extraction}}} dV = -\int_{m_0}^{m} \frac{dm(t)}{m(t)}$$

$$\frac{V_{\text{extraction}}}{V_{\text{reactor}}} = -\ln m - \ln m_0$$

Combining the logarithms, the mass of material remaining in the reactor is obtained as a function of the volume of CO$_2$ used for extraction:

$$m = m_0 \cdot e^{-V_{\text{extraction}}/V_{\text{reactor}}}$$

From the equation above, the mass of material extracted by a given volume of CO$_2$ is obtained:

$$m_0 - m = m_0 - m_0 \cdot e^{-V_{\text{extraction}}/V_{\text{reactor}}}$$

$$m_0 - m = m_0 \left(1 - e^{-V_{\text{extraction}}/V_{\text{reactor}}} \right)$$

(2.11)

Dividing Eq. 2.11 by $m_0$, the amount of material extracted relative to the amount of starting material can be calculated:

$$\frac{m_0 - m}{m_0} = 1 - e^{-V_{\text{extraction}}/V_{\text{reactor}}}$$

(2.12)

An ideal resolution (as defined on p. 47), i.e. a complete, irreversible, equimolar reaction between the racemate and resolving agent is assumed. In this case, the initial mass $m_0$ of the unreacted enantiomeric mixture, as shown in Eq. 2.9, is $m_{\text{rac}} \cdot (1 - mr)$. 

50
Substituting this into Eq. 2.12 and rearranging yields:

\[
\frac{m_0 - m}{(1 - mr) \cdot m_{rac}} = 1 - e^{-\frac{V_{\text{extraction}}}{V_{\text{reactor}}}}
\]

Let \( \hat{m}_e \) denote the expected theoretical mass of the extract \( m_0 - m \). It can be expressed by rearranging the above equation:

\[
\hat{m}_e = (1 - mr) \cdot m_{rac} \cdot \left(1 - e^{-\frac{V_{\text{extraction}}}{V_{\text{reactor}}}}\right)
\]

(2.13)

The mass of the raffinate can be expressed as the sum of three terms: the mass of racemate consumed in an equimolar reaction with the resolving agent \( mr \cdot m_{rac} \), the mass of the resolving agent \( m_{res} \), and the mass of the unreacted enantiomers \( (1 - mr) \cdot m_{rac} \) minus the mass of the extract \( \hat{m}_e \):

\[
\hat{m}_r = mr \cdot m_{rac} + m_{res} + \left[ (1 - mr) \cdot m_{rac} - \hat{m}_e \right]
\]

Expanding the term \( (1 - mr) \) in the above equation, simplifying and grouping terms containing \( m_{rac} \) together yields:

\[
\hat{m}_r = mr \cdot m_{rac} + m_{res} - mr \cdot m_{rac} - \hat{m}_e + m_{res}
\]

\[
\hat{m}_r = m_{rac} - \hat{m}_e + m_{res}
\]

(2.14)

The extract and raffinate yields are calculated simply based on the theoretical masses defined in Eqs. 2.13 and 2.14:

\[
\hat{Y}_e = \frac{m_e}{\hat{m}_e}
\]

(2.15a)

\[
\hat{Y}_r = \frac{m_r}{\hat{m}_r}
\]

(2.15b)

Values of \( \hat{Y} \) are expected to range from 0–1, but both \( \hat{Y}_e \) and \( \hat{Y}_r \) can exceed 1. The conditions under which this occurs are similar to those described on p. 48 for \( \hat{Y} \): values of \( \hat{Y}_e \) can exceed 1 if the diastereomer formation is incomplete or if the dia-
stereomers dissociate, \( \hat{Y}_r \) can exceed 1 if the extraction is less efficient than calculated by the idealized CSTR model.

In a further similarity to \( \hat{Y} \), values of \( \hat{Y} \) become invalid if \( mr \geq 1 \). Due to the term \((1 - mr)\), the value of \( \hat{m}_e \) becomes zero if \( mr = 1 \), causing the value of \( \hat{Y}_e \) to become invalid due to a division by zero. At this point, \( \hat{Y}_r \) is still valid, although \( \hat{m}_r \) is now equal to the entire mass of materials used for the resolution. If \( mr > 1 \), both \( \hat{m}_e \) and \( \hat{m}_r \) become physically invalid by decreasing below zero and by exceeding the mass of available material, respectively. Therefore, as with \( \hat{Y} \), if diastereomer dissociation in a system causes the resolution to proceed at \( mr \geq 1 \), \( \hat{Y} \) cannot be used to describe the yields for the system.

### 2.6.3. Resolution Efficiency

The efficiency of a resolution takes into account both the yields of the products and their optical purities. In the approaches detailed below, optical purity can be described by the general form of enantiomeric excess defined in Eq. 2.4 or averaged ee values obtained by applying Eq. 2.5. For brevity, the following equations will use ee, with the understanding that \( \bar{ee} \) could be used in their place. In cases where \( \bar{ee} \) is used, yields must be calculated from the cumulative masses of the averaged fractions. Note, furthermore, that in the equations that follow, "fraction" and the running index \( i \) are used to mean the extract or the raffinate, not in the sense used for Eq. 2.5.

The resolution efficiency of a single fraction can be described by the selectivity:

\[
\hat{S}_i = \hat{Y}_i \cdot ee_i
\]  

(2.16)

Selectivity is used when only one of the fractions is analyzed. The values of \( \hat{S} \) are expected to vary between 0–1. However, if values of \( \hat{Y} \) exceed 1 (see p. 48) and \( mr \neq 0.5 \), it is theoretically possible for \( \hat{S} \) to exceed 1. Due to the conditions required for this to occur (negligible material losses, enantiomeric excesses approaching 1), it has not been observed in any experiments.

If both the extract and raffinate are evaluated, the overall resolution efficiency can be described by the F parameter, which has two distinct definitions. First, if yields are characterized by \( Y \), the F parameter is calculated from the yields and optical purities of the extract and raffinate (indices \( e \) and \( r \), respectively) by the following equation:

\[
F = Y_e \cdot ee_e + Y_r \cdot ee_r
\]  

(2.17)
Because ee values are always between 0–1, and because the definition of $Y$ ensures that $Y_e + Y_r \leq 1$, values of $F$ always range from 0 to 1.

The second definition of the $F$ parameter is used when yields are described by $\hat{Y}$. As with Eq. 2.17, the indices $e$ and $r$ refer to the extract and raffinate, respectively.

$$\hat{F} = \frac{\hat{Y}_e \cdot ee_e + \hat{Y}_r \cdot ee_r}{2}$$  \hspace{1cm} (2.18)

Unlike $Y$, values of $\hat{Y}$ do not sum to 1, ranging instead from 0 to 1 (and possibly exceeding 1, see p. 51). The division by 2 therefore aims to bring the values of $\hat{F}$ between 0–1 in order to facilitate comparison with $F$. Values of $\hat{F}$ could potentially exceed 1, provided that $\hat{Y} > 1$ in the extract or raffinate, $mr \neq 0.5$, enantiomeric excesses are almost equal to 1 and material losses are negligible. As with $\hat{S}$, this has not been observed in any experiments.
3. RESULTS AND DISCUSSION

The results presented in the following sections have been obtained in several concurrent, ongoing research topics. These topics are grouped by racemate–resolving agent pairing and by resolution technique, and as such, the order in which they appear in this thesis does not necessarily reflect the chronological order in which the experiments have been carried out.

3.1. RESOLUTION OF IBUPROFEN WITH 1-PHENYLETHANAMINE

This resolution system has been investigated previously by Molnár [184], using extraction with supercritical carbon dioxide from a packed column. In these experiments (similar to the in vacuo technique described in 2.3.2), the racemate and resolving agent were dissolved in an organic solvent along with an inert support, and the solvent was evaporated under vacuum. During evaporation, the diastereomers had formed, and the subsequent extraction only served to separate them from the unreacted enantiomeric mixture. Although the preparation method was executed under identical conditions for all experiments, Molnár’s results showed that the pressure of extraction affects optical purities both in the extract and raffinate. These findings suggested that the components undergo a reaction in scCO$_2$, and that the in situ approach could be viable (Section 3.1.1).

Antisolvent resolutions of this system have also been investigated. The results of experiments using the GAS method are presented in Section 3.1.2. Preliminary results of transferring the resolution to the SAS technique are given in Section 3.1.3.

Due to the detailed nature of the investigation Molnár carried out on this system using packed column extraction, the highly similar in vacuo technique was not investigated.

3.1.1. In situ METHOD

In these experiments, unless otherwise noted, 200.0±0.5 mg (0.97±0.002 mmol) solid racemic ibuprofen and 58.7±0.5 mg (0.49±0.004 mmol, mr = 0.5) liquid (R)-(+)-1-phenylethanolamine was measured into the high-pressure reactor. The liquid trap was filled with 30 ml methanol. The ideal CSTR model was applied, thus yields were characterized by $\hat{Y}$ and the resolution efficiency was characterized by $\hat{F}$. During the extraction step, the amount of CO$_2$ used for washing was approximately twice the
Figure 19: Results of the in situ experiments carried out on the IBU–(R)-PhEA resolution system (at $T = 40 \, ^\circ\text{C}$). Fitted quadratic surface shown as contour lines spaced 0.02 dimensionless units apart, thick contour lines indicate 0.1 dimensionless unit. Dotted lines mark the distance of data points from the surface. Dashed lines only indicate general trends and are not the result of mathematical modelling. For supplementary data, see Table A1 (Appendix C, p. A-4).

The effect of pressure and reaction time was studied at three pressure levels (100 bar, 150 bar, 200 bar) and in the range of 1–100 h at 40 °C. The resolution efficiency was calculated for each experiment, the results are shown in Figure 19 (each point corresponds to a single experiment).

The pressure and reaction time both exert a positive influence on the resolution efficiency within the investigated range of values. However, interactions between the two factors cause the effect of pressure to diminish towards lower values of reaction time and vice versa, to the extent that at the lower limits of the two factors, almost no effect is exerted. This can be observed in Figure 19: at $p = 100$ bar the F parameter stagnates around 0.2, and at $t = 1$ h, it only increases from 0.2 to approximately 0.25. Increasing the reaction time amplifies the effect of pressure on the resolution efficiency, i.e. at longer reaction times, $\hat{F}$ increases more sharply towards higher pressures. Similarly, at higher pressures, the resolution efficiency shows a sharper increase towards longer reaction times.

Both of these effects suggest that the diastereomer forms in an equilibrium reac-
Figure 20: In situ resolution of IBU with (R)-PhEA. Optical purities in the CO$_2$ phase (at $p = 200$ bar). Dashed lines only indicate a general trend and are not the result of mathematical modelling. Horizontal axis extended into negative values for clarity. For supplementary data, see Table A2 (Appendix C, p. A-5).

The changes in the F parameter shown in Fig. 19 are largely due to changes in the enantiomeric excess values. The effect of pressure on optical purities in particular is explained by the differing crystallographic unit cell sizes of the diastereomeric salts [79]. The (R,R)-diastereomer has a unit cell volume of 1997.2 Å, while the (S,R)-diastereomer has a unit cell volume of 2005.2 Å. Increasing the pressure favors the formation of the diastereomeric salt with the smaller volume, thus the raffinate should be enriched in the (R,R)-diastereomer at higher pressures. Measured optical purities are in agreement with this predicted trend.

The effect of temperature was investigated between 40 °C and 60 °C at $p = 200$ bar, at reaction times ranging from 1 h to 130 h. Reactions were sampled at
different intervals, so that the ee in the CO\textsubscript{2} phase could be tracked. Analytics indicate that, compared to the amount of IBU extracted from the reactor, PhEA is only removed in negligible quantities during sampling. Although this increases mr, it was found \cite{184} that mr does not exert a significant effect between 0.45–0.65. Therefore, the initial mr for these sampled experiments was set to 0.45 by decreasing the amount of PhEA to 52.9±0.5 mg (0.44±0.004 mmol), and the number of samples, as well as the volume of CO\textsubscript{2} used for sampling were controlled such that mr would not increase above 0.65. The results from multiple experiments carried out at 40 °C and 50 °C are shown in Figure 20. Above 50 °C, the resolution could not be carried out: the raffinate was liquid rather than crystalline, and the enantiomeric excesses of both the extract and the raffinate were near zero.

As Fig. 20 shows, optical purity in the CO\textsubscript{2} phase increases according to a saturation curve. Increasing the temperature raises both the initial slope of the curve (this can be assumed based on the markedly different values of ee at 1 h), as well as the saturation value. These observations are consistent with an equilibrium reaction: higher temperatures increase the reaction rate (according to the Arrhenius equation) and – provided that the enthalpy of formation for the diastereomers is positive – shift the equilibrium in the forward direction (according to the van ’t Hoff equation).

3.1.2. GAS METHOD

In these experiments, unless otherwise noted, 150±0.5 mg (0.73±0.002 mmol) solid racemic ibuprofen and 44.0±0.5 mg (0.36±0.004 mmol, mr = 0.5) liquid (R)-(+)\textsubscript{-}1-phenylethanamine was dissolved in 2 ml MeOH and measured into the high-pressure reactor. After reaching the desired reaction pressure during the filling phase, the reactor contents were stirred for 1 h before the CO\textsubscript{2}-soluble compounds were extracted (see Section 2.3.1). The liquid trap was filled with 30 ml MeOH. When using the GAS technique, washing the reactor with scCO\textsubscript{2} extracts organic solvent (in this case, methanol) along with soluble components (unreacted IBU, in this case). This causes the solvent properties (most importantly, the solvent polarity and thereby the solvent power) to vary continuously as a function of the volume of CO\textsubscript{2} used for washing. Under these conditions, the idealized CSTR model no longer applies. Furthermore, as detailed later, significant dissociation of the diastereomeric salts causes the resolution to also proceed at mr > 1, thus the definitions based on ideal resolution (\(\hat{Y}, \hat{F}\)) cannot be used either. Therefore, for these experiments, yields were calculated using the definition based on the total amount of starting material Y (Eq.
Figure 21: GAS resolution of IBU with (R)-PhEA. Optical purities in the raffinate and extract at 45 °C. For supplementary data, see Table A3 (Appendix C, p. A-5).

Figure 22: GAS resolution of IBU with (R)-PhEA. Resolution efficiency at 45 °C. For supporting data, see Table A3 (Appendix C, p. A-5).

2.6). Accordingly, the resolution efficiency was characterized by $F$ (Eq. 2.17).

The effect of pressure was investigated between 100–210 bar at 45 °C. For each experiment, ee and $Y$ were calculated for both the extract and raffinate, optical purities for the extract and raffinate are shown in Figure 21. In the range investigated, pressure exerts virtually no effect on the optical purity of the raffinate, while that of the extract shows a decreasing trend as the pressure increases. The effects of pressure on the ee values result in a steady decrease in resolution efficiency as pressure increases, shown in Figure 22.
Since the above trend indicated that decreasing the pressure raises the resolution efficiency, experiments at 90 bar were attempted. However, these experiments suffered from poor reproducibility and generally did not yield results consistent with the trend described above.

The effects shown in Figs. 21 and 22 can be explained by a decomposition of the diastereomeric salts (into the constituent acid and amine) in an equilibrium reaction: the raffinate formation does not proceed to completion, and during the washing phase, gradual removal of IBU and PhEA from the reactor vessel further shifts the equilibrium towards decomposition. This decreases the yield of the raffinate and the optical purity of the extract (due to the antipode leaching out of the reactor). It would seem reasonable to conclude that the decomposition is more pronounced at higher pressures because of the increased solvent power of carbon dioxide due to its increased density. However, there is another factor influencing solvent power in the reactor: the relative amounts of organic solvent and carbon dioxide. Since polar organic solvents act as an entrainer, higher MeOH:CO\(_2\) ratios increase the solvent power. Since the amount of methanol was the same for all experiments discussed so far, and since establishing higher pressures in the reactor required higher amounts of carbon dioxide, the relative amount of methanol decreased with increasing pressure. This decrease in solvent power acts against the effect of increased CO\(_2\) density, and only their net effect is observed. Therefore, additional experiments were carried out to study the effect of the antisolvent-to-solvent ratio independent of the pressure.

The effect of the antisolvent:solvent mass ratio \(R\) on the diastereomeric salts was investigated at 150 bar and 45 °C by varying the amount of solvent between 1–4 ml (compared to 2 ml in other experiments), while keeping the masses of IBU and PhEA constant. Additionally, the effect of using ethanol instead of methanol was studied. In one experiment, a mixture of 1 ml EtOH and 1 ml MeOH was used, this is denoted in the following figures as "EtOH+MeOH".

Figure 23 shows the effect of \(R\) on the raffinate yields for the two solvents (EtOH and MeOH) as well as the solvent mixture. Both the ethanol and methanol exhibit the same general trend: yields increasing along with increasing \(R\), according to a saturation curve with a roughly linear initial section. For ethanol, no data points above \(R = 15:1\) are available, as the relatively lower solubility of IBU and PhEA in this solvent would have resulted in insufficient amounts of raffinate and extract had the solvent amount been reduced significantly below 2 ml (corresponding to an approximate \(R\) of 14:1). Yields for experiments with ethanol are consistently higher.
Figure 23: GAS resolution of IBU with (R)-PhEA. Yields in the raffinate at 150 bar and 45 °C. Dashed lines only indicate general trends and are not the result of mathematical modelling. For supplementary data, see Table A4 (Appendix C, p. A-6).

Figure 24: GAS resolution of IBU with (R)-PhEA. Yields in the raffinate at 150 bar and 45 °C. For supplementary data, see Table A4 (Appendix C, p. A-6).

than those with methanol, with the 1:1 mixture of the two solvents situated between the two series (due to the similar densities of ethanol and methanol, both the experiments with 2 ml MeOH and EtOH, as well as the experiment with 1 ml MeOH and 1 ml EtOH, appear around \( R = 14:1 \)). The similarity of the trends is further accentuated if the yields are plotted against the \( \text{CO}_2: \text{solvent molar ratio} \) \( R_m \), shown in Figure 24. Although experiments with methanol appear to have slightly lower yields, both solvent series follow the same saturation curve, with the initial section having less pronounced linearity. Note furthermore that the experiment with the solvent mixture
Figure 25: GAS resolution of IBU with (R)-PhEA. Optical purities in the raffinate at 150 bar and 45 °C. For supplementary data, see Table A4 (Appendix C, p. A-6).

also falls on the same saturation curve.

The effect of $R$ on the enantiomeric excess in the raffinates is shown in Figure 25. One data point has been omitted from this graph: the experiment in the MeOH series at $R = 8.7:1$. As can be seen in Fig. 23, the raffinate yield in this experiment was extremely low: there was virtually no diastereomer to recover. The determination of enantiomeric excess from such a low sample mass is unreliable, and it was thus omitted from Fig. 25 (it was, however, included in Fig. 23 as the low yield was pertinent information for that graph). Although the solvent ratio does not seem to influence the optical purities significantly, the choice of solvent does exert some effect on the ee values. Experiments conducted with ethanol have raffinate ee values between 0.8–0.9, while experiments with methanol – with one exception – show raffinate ee values between 0.6–0.8. The raffinate optical purity obtained using the solvent mixture appears to lie closer to the range of the EtOH experiments.

Experiments studying the effect of pressure (see p. 58) found no clearly identifiable trends in the behavior of the yields. In these experiments, $R$ (calculated for methanol) varied from approximately 11:1 at 100 bar to approximately 18:1 at 200 bar. Although Fig. 23 shows a significant variation of yields for methanol in this range of $R$, in the earlier experiments the effect of $R$ cannot be extricated from the effect of pressure. The density of carbon dioxide varied between approximately 0.50–0.80 g/ml for these experiments, which might exert a strong enough effect on the solvent power of CO$_2$ to mask the effect of $R$. 

61
Figure 26: GAS resolution of IBU with (R)-PhEA. SEM images of raffinates obtained from EtOH at 150 bar and 45 °C. Brightness and contrast adjusted for clarity, unprocessed images are included in Figure A1 (Appendix B, p. A-3).
The structure of the diastereomers was studied by scanning electron microscopy and powder X-ray diffraction. Figure 26 shows SEM images from two raffinates prepared from ethanol at different values of $R$. At $R = 9.5:1$, the solid phase is composed of irregular bladed crystals of various sizes. At $R = 14.4:1$, two distinct crystalline phases are visible: a tightly packed collection of cylindrical crystals (seen to the right side of Fig. 26b) and loosely arranged, fibrous crystals with very high length:diameter ratios. Figure 27 shows the diffractograms of the same two raffinates. Both the relative intensities and positions of the peaks show an almost exact correlation, indicating that the crystallographic structure is the same for both diastereomers. Similar analyses carried out for the raffinates prepared from methanol confirm that $R$ influences the habit (i.e. the size and shape) of the diastereomeric crystals, without altering their crystallographic structure [185].

The effect of the molar ratio (mr) was investigated at 130 bar and 45 °C using 2 ml MeOH, by keeping the mass of IBU constant at 150 mg, and altering the mass of PhEA between the different experiments, such that mr was varied between 0.3–1.25. Note that, since IBU and PhEA react in a 1:1 stoichiometric ratio, mr = 1 corresponds to the equivalent molar ratio. An "ideal resolution" (in the sense explained on p. 47), i.e. one based on a complete and irreversible reaction between the racemate and the resolving agent, could not be carried out at or above the equivalent molar ratio. At this mr, the resolving agent would bind the entire mass of the racemate, causing the yield of the extract to drop to zero, while the diastereomers – being racemic – would have an ee of 0, resulting in a resolution efficiency that is also zero. The
fact that successful resolutions have been performed at molar ratios of 1 and 1.25 (at and above the equivalent mr, respectively) indicates that the assumption of a perfect resolution does not hold. This is the rationale behind using $Y$ and $F$ for these experiments instead of $\hat{Y}$ and $\hat{F}$.

Figure 28 shows the effect of mr on the extract and raffinate yields. At low molar ratios ($mr < 0.6$, the extract and raffinate yields show opposite linear trends: extract yields decrease from 0.9 to 0.25, while raffinate yields increase from 0.2 to 0.4. Due
to the definition of $Y$, its values must necessarily remain between 0 and 1, thus the linear trends cannot continue indefinitely. Raffinate yields are expected to reach 0 approximately at $mr = 0.2$, the same value where extract yields appear to reach 1. Above $mr = 0.6$, the linear trends diminish and the yields appear to take on constant values independent of $mr$. Extract yields stabilize around 0.2, raffinate yields appear to stabilize between 0.6–0.7.

The effect of $mr$ on enantiomeric excess values is shown in Figure 29. Extract ee values appear to exhibit a similar trend as was observed for yields: a roughly linear increase from 0.1 to 0.3 for $mr < 0.6$, becoming less pronounced and giving way to constant values around 0.55. The linear section appears to reach 0 at approximately $mr = 0.2$. The raffinate ee values, however, show a different trend: for experiments where $mr < 0.6$, values appear to be constant at around 0.75. For higher $mr$ although the variation between points is significant, the values vary around 0.2.

The aggregate effects of $Y$ and ee on the resolution efficiency are shown in Figure 30. The opposing linear trends in yields, as well as the trends in enantiomeric excesses create a maximal trend for $mr$ values between 0.3–0.6. Towards higher values of $mr$, both yields and enantiomeric excesses assume constant or nearly constant values, resulting in the resolution efficiency stabilizing around 0.2. Although not observed directly, it is expected that around $mr = 0.2$, due to raffinate yield and extract ee

**Figure 30:** GAS resolution of IBU with (R)-PhEA. Efficiency of the resolution at 130 bar and 45 °C. Solid marker indicates experiment calculated with estimated extract yield. Blue dotted line indicates expected trend for non-dissociating diastereomer. Red dotted line indicates resolution efficiency that establishes above $mr = 1$. Dashed line indicates general trend of $F$ values. For supplementary data, see Table A5 (Appendix C, p. A-6).
decreasing to 0, the overall resolution efficiency will also decrease to 0.

The effects described above indicate that a decomposition of the diastereomeric salts occurs, as the trends exhibited by both the yields and ee values (and, consequently, the resolution efficiency) are incompatible with irreversible diastereomer formation.

If such an irreversible reaction is assumed, yields would vary in a linear fashion between \( mr = 0 \) and \( mr = 1 \). When no resolving agent is added (\( mr = 0 \)), the entire mass of the racemate would – in theory – be extracted, causing \( Y \) in the extract and raffinate to be 1 and 0, respectively. Conversely, at \( mr = 1 \), the entire mass of racemate is precipitated, thus \( Y \) in the extract becomes 0, with \( Y \) in the raffinate equal to 1. The fact that a constant, measurable amount of extract was recovered for \( mr \geq 1 \) suggests that diastereomer decomposition allows a certain amount of IBU to be extracted, regardless of mr. Diastereomer decomposition also explains why the observed trends seem to have intercepts at \( mr = 0.2 \), rather than \( mr = 0 \): if small quantities of resolving agent are added, the formed diastereomers decompose entirely, and raffinate is only recovered above a certain threshold value of mr(0.2 in this case).

Assuming an irreversible diastereomer formation, the exact trends of ee cannot be predicted, as these depend on the specific racemate–resolving agent interaction. However, certain values at \( mr = 0 \) and \( mr = 1 \) can be given: if no resolving agent is added (\( mr = 0 \)), the racemate is extracted entirely, leading to an extract ee of 0. If \( mr = 1 \), the racemate remains in the raffinate and thus ee = 0 in the raffinate is expected. In contrast with these theoretical expectations, observed raffinate ee values at \( mr \geq 1 \) are not zero, suggesting that the \((S)\)-IBU–\((R)\)-PhEA diastereomer decomposes more readily. Furthermore, the linear trend of extract ee values has its expected intercept at \( mr = 0.2 \), i.e. the extract becomes racemic at this mr value (rather than \( mr = 0 \)). This further indicates that at low values of mr, the diastereomers decompose entirely, causing the extract ee (and raffinate yield) values to drop to 0.

Based on the theoretical considerations above, the resolution efficiency would exhibit a maximal trend: at \( mr = 0 \), a value of 0 is expected for the raffinate yield and extract ee, while at \( mr = 1 \), zero values are expected for the extract yield and raffinate ee. The value of \( F \) at the maximum and the value of mr where the maximum occurs both depend on the specific racemate–resolving agent interaction, however, the maximum generally occurs near the half-equivalent molar ratio [79, 186]. The
expected trend for $F$ when no diastereomer decomposition occurs is shown as a blue dotted line in Fig. 30. Compared to this theoretical trend, the actual observed values show two major discrepancies: the value of observed $F$ is expected to reach 0 at $mr = 0.2$ rather than $mr = 0$, and $F$ does not go approach 0 when $mr$ is increased to and above 1, stabilizing instead at a constant value (shown in Fig. 30 as a red dotted line). Both of these effects can be attributed to diastereomer decomposition: at $mr \leq 0.2$, diastereomers decompose completely, leading to the anomalous intercept, while at high $mr$, a fixed amount of diastereomer is decomposed, preventing $F$ from reaching 0.

The atypical behaviour of the raffinates hinted at the possibility of producing ibuprofen in higher purity and with better yield than is obtainable in traditional resolutions in organic solvents. The eutectic phase behaviour of IBU results in a maximum purity of ee = 0.88 [187], circumventable only by derivatization, leading to additional losses. Further experiments focused on the possibility of exceeding this limit by submitting the products of a GAS resolution to a second GAS step. To this end, the effects of starting from non-racemic ibuprofen were investigated. This was done by raising the initial ee of ibuprofen with the addition of (S)-IBU, and switching the resolving agent to (S)-PhEA. Switching the resolving agent was necessary because (R)-PhEA reacts preferentially with (R)-IBU, as evidenced by the excess of (R)-IBU in the raffinates. Thus, according to the Marckwald principle, (S)-PhEA would react preferentially with (S)-IBU, and as such would be more suitable for resolving IBU mixtures with high (S)-enantiomer content.

The effect of the initial enantiomeric excess ee$_0$ was investigated at 130 bar, 45 °C and $mr = 0.5$ by mixing the starting racemic ibuprofen with varying amount of (S)-IBU so that ee$_{(S)}$ was varied between 0–0.8. The initial optical purity of ibuprofen exerts no significant influence on the yields of either the extract or the raffinate: they vary between 0.4–0.6 and 0.3–0.5, respectively, with no apparent trend.

The effect of ee$_0$ on the optical purities of the extract and raffinate is shown in Figure 31 (note that by using (S)-PhEA, raffinates are enriched in (S)-IBU). At ee$_0 = 0$, the data points have been estimated from resolutions with (R)-PhEA (according to the Marckwald principle, the magnitudes of the ee values should be equal, with signs inverted). These correspond a "traditional" resolution: resolving agent added in half-equivalent molar ratio to a racemate, inducing a separation of the enantiomers (by preferentially binding (S)-IBU in the raffinate). On the other hand, ee$_0 = 1$ would involve "resolving" enantiopure (S)-IBU, resulting in an ee of 1 for both the extract and
raffinate (irrespective of their yields). Both the extract and raffinate optical purities increase along with ee₀, seemingly without the limitation of ee = 0.88 due to a eutectic point: at ee₀ = 0.776, the raffinate ee exceeds 0.9 (R)-IBU. Around ee₀ = 0.3, the extract ee reaches zero: at this point, the initial fraction of (S)-IBU has increased to the point that (R)-IBU is no longer in excess in the extract.

An additional experiment has been performed to study the effect of the molar ratio at nonzero ee₀. This was done in order to assess the feasibility of subjecting raffinates to direct purification: i.e. carrying out an antisolvent resolution on the diastereomeric salts that formed in a previous resolution experiment. In this instance, the second resolution would be performed at mr = 1 and at a – presumably nonzero – ee₀ corresponding to the raffinate ee of the first resolution. Table 2 summarizes the result of this experiment compared to a control experiment performed at half-equivalent molar ratio.

The optical purity in the raffinate is not affected by the increase in the molar ratio. This phenomenon is explained by the fact that ee₀ = 0.776 corresponds to a (S)-IBU fraction of 0.888 (note that, due to the equal molar masses of the enantiomers, mass fraction and mole fraction are numerically equal). Thus, at mr = 0.5, (S)-

---

Figure 31: GAS resolution of IBU with (S)-PhEA. Optical purities at 130 bar and 45 °C, mr = 0.5. Solid markers indicate estimates based on resolutions with (R)-PhEA. Solid black line indicates ee = ee₀. Dashed lines only indicate general trends and are not the result of mathematical modeling. For supplementary data, see Table A6 (Appendix C, p. A-7).
Table 2: GAS resolution of IBU with (S)-PhEA. Optical purities of the raffinate and extract (indices r and e, respectively), at 130 bar, 45 °C and ee₀ = 0.776.

<table>
<thead>
<tr>
<th>mr [-]</th>
<th>0.513</th>
<th>0.891</th>
</tr>
</thead>
<tbody>
<tr>
<td>eeᵣ [-]</td>
<td>0.93</td>
<td>0.93</td>
</tr>
<tr>
<td>eeₑ [-]</td>
<td>0.76</td>
<td>0.36</td>
</tr>
</tbody>
</table>

PhEA is in roughly 75% excess at mr = 0.513, and roughly equimolar with (S)-IBU at mr = 0.891. Taking into account the decomposition of the raffinate, this is the reason behind the raffinate ee remaining unchanged between the two molar ratios. Also, as the mr increases, the fraction of (R)-IBU in the extract increases as more (S)-IBU is bound in the raffinate by (S)-PhEA, explaining the decrease in the extract ee. The most important conclusion drawn from these results is that the initial ee does not influence the raffinate optical purity, hinting at the possibility of direct raffinate purification. This was eventually realized using a raffinate obtained with the SAS technique, see Section 3.1.3.

3.1.3. SAS METHOD

Two preliminary experiments performed at 130 bar and 45 °C have been successful in transferring the resolution to the SAS technique. Additionally, the raffinate of one experiment has been subjected to direct purification by using it as starting material for a GAS experiment (with no further resolving agent added).

Experiments have been performed according to the steps described in Section 2.4.2: the starting materials have been dissolved in methanol, then injected into the crystallizer vessel which was pressurized by a continuous flow of scCO₂. The amounts of IBU, (R)-PhEA and MeOH are given in Table 3, along with the results of the experiments. As the resolution could be carried out at mr ≈ 1, the ideal resolution model cannot be applied, thus yields were characterized by Y (Eq. 2.6).

Enantiomeric excesses in both the extract and raffinate are comparable to those obtained with the GAS method. Although at mr = 1, the high volume of MeOH required to completely dissolve the starting materials led to a significantly lower concentration, yields did not decrease drastically compared to those obtained with the GAS method.

Direct purification of the raffinate obtained from the equimolar SAS experiment was carried out by a secondary GAS step: 118.7 mg of the raffinate was dissolved in 2 ml MeOH (estimated masses assuming mr = 1: 74.8 mg IBU, 43.9 mg (R)-PhEA).
Table 3: SAS resolution of IBU with (R)-PhEA. Results of the two preliminary experiments ($p = 130$ bar, $T = 45^\circ\text{C}$).

<table>
<thead>
<tr>
<th></th>
<th>Step 1</th>
<th>Step 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>racemic IBU [g]</td>
<td>1.45</td>
<td>1.44</td>
</tr>
<tr>
<td>(R)-PhEA [g]</td>
<td>0.43</td>
<td>0.85</td>
</tr>
<tr>
<td>mr [-]</td>
<td>0.505</td>
<td>1.005</td>
</tr>
<tr>
<td>methanol [ml]</td>
<td>7.5</td>
<td>20</td>
</tr>
<tr>
<td>extract ee(S) [-]</td>
<td>0.230</td>
<td>0.316</td>
</tr>
<tr>
<td>raffinate ee(R) [-]</td>
<td>0.544</td>
<td>0.506</td>
</tr>
<tr>
<td>extract Y [-]</td>
<td>0.394</td>
<td>0.239</td>
</tr>
<tr>
<td>raffinate Y [-]</td>
<td>0.321</td>
<td>0.226</td>
</tr>
</tbody>
</table>

Figure 32: Antisolvent resolution and purification of IBU with (R)-PhEA. Optical purities at 130 bar and 45 °C. Step 1: $ee_0 = 0$, mr ≈ 1. Step 2: $ee_0 = 0.506$ (R)-IBU, mr ≈ 1. For supplementary data, see Table A7 (Appendix C, p. A-7).

and subjected to a GAS resolution at 130 bar and 45 °C. The second step can be considered equimolar, with ee$_0$ equal to the raffinate ee in the first step.

Figure 32 shows the optical purities for the equimolar SAS experiment and for the GAS direct purification of its raffinate. The raffinate, possessing an enantiomeric excess of 0.506 (R)-IBU (corresponding to ee$_0 = 0.506$ (R)-IBU in the second step), was resolved into a nearly racemic extract (ee$_{(R)} = 0.068$) and a raffinate of high purity (ee$_{(R)} = 0.900$). Thus, in terms of the enantiomeric excesses, the second step approaches a perfect resolution (nonetheless, since it was achieved at approximately mr = 1, the conditions of a "ideal resolution" as described in 2.6.2 on p. 47 do not hold true).

In both experiments, yields were comparable to earlier GAS experiments carried
out at various values of mr and ee$_0$. Overall, the two-step procedure yielded 30.1 mg nearly racemic extract and 55.5 mg high-purity raffinate from 118.7 mg of the SAS raffinate, i.e. 72.1% of the material loaded into the reactor was recovered. The main limiting factor in the second step was the reactor capacity, i.e. the amount of material that can be resolved in a single batch. Future experiments could make use of the higher capacity of the SAS equipment, resolving the raffinate in a secondary SAS step.
3.2. Resolution of cis-permethric acid with \((S)-(+)-2-(N\text{-benzylamino})\text{butan-1-ol}\)

The \textit{in situ} technique was successfully applied to this resolution system, detailed results are presented in Section 3.2.1.

The \textit{in vacuo} method was also applied to the system. Optical purities in the extract, determined by sampling the scCO\(_2\) phase, were similar to those obtained with the \textit{in situ} experiments. Due to the advantages of the \textit{in situ} technique (chiefly, the elimination of organic solvent use), focus was shifted away from the \textit{in vacuo} method, and it was not investigated further.

Preliminary experiments using the GAS method failed to produce diastereomer crystals. Analyses of the recovered extract solution and liquid raffinate indicated that no chiral discrimination occurred, as both fractions were racemic.

3.2.1. \textit{In situ} Method

In these experiments, unless otherwise noted, 400.0±0.5 mg (1.91±0.002 mmol) of solid racemic cPA and 158.9±0.5 mg (0.86±0.003 mmol, 97.1% purity, \(m_r = 0.45\)) solid \((S)\text{-BAB}\) were measured into the reactor vessel. The initial \(m_r\) was chosen as 0.45 as opposed to 0.5 because prior to the washing phase, the scCO\(_2\) phase was sampled three times during the reaction’s runtime, and – as explained in Section 3.1.1 – this increases \(m_r\). Although the CSTR model could be applied to each sampling as well as the final washing, empirical evidence suggested that at the low CO\(_2\) volumes used during the samplings, the effects of nonidealities (dead volumes, flow rate variations, material losses) become significant enough to cause large differences between the actual extracted masses and those suggested by the idealized model. For this reason, the definitions of \(\hat{Y}\) and \(\hat{S}\) were used (see Section 2.6.2 Eq. 2.7 and Section 2.6.3 Eq. 2.16). The liquid trap was filled with 30 ml MeOH.

The effect of reaction time \(t\) on the extract optical purities was studied in the range of 1–50 h. Figure 33 shows the enantiomeric excess values in the scCO\(_2\) phase as determined by sampling (i.e. each data point corresponds to one sampling in an experiment). Although the extract ee does increase along with the reaction time, this approximately 50% increase (from \(~0.18\) to \(~0.28\) \((+)-cPA\)) requires a 4200% increase in reaction time (1 h to 43 h). This indicates that the diastereomer formation reaction either proceeds to a large extent within 1 h and slows down significantly thereafter, or it goes to completion within 1 h followed by a slow rearrangement. For
Figure 33: *In situ* resolution of cPA with (S)-BAB. Optical purity in the scCO₂ phase at 200 bar, 45 °C. For supplementary data, see Table A8 (Appendix C, p. A-7).

![Graph](image)

Figure 34: *In situ* resolution of cPA with (S)-BAB. Raffinate enantiomeric excess and yield at 45 °C. For supplementary data, see Table A9 (Appendix C, p. A-8).

this reason, experiments detailed below were carried out at 1 h reaction time.

The effect of pressure was studied between 150–215 bar at 45 °C. Figure 34 shows the extract optical purities and yields for the individual experiments (i.e. optical purities obtained by sampling are not shown). Raffinate yields exhibit no discernible trend: the variation between the data points over the pressure range investigated was less than 0.1 (cf. typical yield values of 0.6). On the other hand, the enantiomeric excesses vary in a wide range: there is a slight increasing trend between 170–215 bar (up to ee(−) = 0.781, at 215 bar), with values dropping off sharply below 170 bar. At
160 bar, the raffinate enantiomeric excess drops to $ee_{(-)} = 0.415$, while at 150 bar, the extract and raffinate are racemic to within detection error. This, coupled with fact that a significant amount of raffinate was obtained at 150 bar, indicates that a diastereomer formation reaction does occur, but without chiral discrimination.

Figure 35 shows the diffractogram of the raffinate obtained at 150 bar, compared against diastereomeric standards prepared by vacuum evaporation, both from $(-)$-cPA and $(+)$-cPA. With the exception of the first peak, just below 9° (which could be missing due to peak broadening or the overall smaller intensity of the raffinate diffractogram), all peaks exhibited by either of the standards also clearly appear on the raffinate diffractogram. This indicates that the raffinate at 150 bar is a physical mixture of the diastereomers, i.e. both diastereomeric salts form at roughly the same reaction rate.

XRD analysis of the raffinate obtained at 200 bar showed that despite its high optical purity ($ee_{(-)} = 0.678$), its crystal structure is different from that of the $(-)$-cPA–$(S)$-BAB standard prepared by vacuum evaporation (see Figure A2 in Appendix C p. A-8). One explanation for this phenomenon is kinetic versus thermodynamic control: the relatively slow (~1 h) formation of the in situ raffinate is likely governed by ther-
modynamic control, favouring a different crystalline structure than the comparatively fast (on the order of minutes) crystallization under vacuum. Another possible cause for the discrepancy in crystal structures is the different solvents from which the samples have been obtained (methanol for the atmospheric standards, scCO\textsubscript{2} for the \textit{in situ} samples).

The effect of temperature on the formed diastereomeric salts was investigated at 200 bar, by conducting three experiments at 35 °C, 45 °C and 55 °C. The results of the experiments are summarized in Table 4. Although yields are only slightly affected by the temperature – showing a small negative trend with increasing temperature – the drastic decrease in the raffinate optical purity at higher temperatures means that, of the temperatures investigated, 35 °C seems to be optimal in terms of raffinate selectivity.

<table>
<thead>
<tr>
<th>T [°C]</th>
<th>ee\textsubscript{(-)} [-]</th>
<th>Y [-]</th>
<th>S [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>0.750</td>
<td>0.537</td>
<td>0.403</td>
</tr>
<tr>
<td>45</td>
<td>0.607</td>
<td>0.559</td>
<td>0.339</td>
</tr>
<tr>
<td>55</td>
<td>0.221</td>
<td>0.461</td>
<td>0.102</td>
</tr>
</tbody>
</table>

Table 4: \textit{In situ} resolution of cPA with (S)-BAB. Optical purity, yield and selectivity in the raffinate at 200 bar.
3.3. **Resolution of cis-permethric acid with (R)-(−)-1-phenylethanamine**

The GAS resolution method was successfully applied to this system, the results are given in Section 3.3.1. The resolution was also transferred to the SAS technique, the results of these experiments are presented in Section 3.3.2.

* cis-Permethric acid could be resolved with PhEA using the *in vacuo* method. Sampling the scCO$_2$ phase at reaction times $>$ 1 h revealed no change in the optical purity, remaining around 25% at 200 bar and 45 °C. Since much higher ee values could be achieved using the GAS method, this technique was not investigated further.

The *in situ* method was attempted but no resolution could be achieved. While reactions at 200 bar and 45 °C yielded large amounts of diastereomer ($Y > 0.8$), no enantioselectivity was observed ($ee_{(−)} < 0.1$) for reaction times between 1–50 h. XRD measurements did not indicate presence of PhEA carbamate in the raffinate, suggesting that the reaction between cPa and PhEA does take place. The lack of optical purity indicates that the *in situ* diastereomer formation is not stereoselective.

### 3.3.1. **GAS Method**

In these experiments, unless otherwise noted, 130±0.5 mg (0.62±0.002 mmol) racemic cPA and 37.6±0.5 mg (0.31±0.004 mmol) (R)-PhEA were dissolved in methanol (2±0.008 ml) and loaded into the reactor. The liquid trap was filled with 30 ml methanol. As detailed later, only the formed diastereomeric salts were evaluated, therefore only raffinate yields were calculated. Due to the reasons presented in Section 3.1.2, the CSTR model cannot be applied to GAS experiments, therefore, raffinate yields and selectivities were characterized by $\tilde{Y}$ (Eq. 2.7b) and $\tilde{S}$ (Eq. 2.16), respectively.

The effect of pressure on the diastereomeric salts was studied between 100 bar and 200 bar at 45 °C. After filling the reactor vessel with CO$_2$ to the desired pressure, its contents were stirred for 1 h to ensure complete precipitation before the extractive washing began (see Section 2.3.1). For each experiment, the raffinate yields and enantiomeric excesses were calculated, these are shown in Figure 36.

In terms of enantiomeric excess, the diastereomers show three distinct regions: virtually racemic ($ee_{(−)} < 0.05$) between 100–120 bar, excellent enantiomeric excess ($ee_{(−)} > 0.8$) or almost enantiopure ($ee_{(−)} = 0.940$, at 150 bar) between 130–170 bar, and poor but definite optical purity ($0.2 < ee_{(−)} < 0.4$) between 180–200 bar. The
raffinate yields decrease more or less continuously from between 100–170 bar from moderate to limited (from $\hat{Y} = 0.608$ at 100 bar to $\hat{Y} = 0.279$ at 170 bar), then drop sharply to almost zero ($\hat{Y} < 0.1$) between 180–200 bar. The aggregate of these effects on the raffinate selectivity is shown in Figure 37.

As can be expected from the definition of the raffinate selectivity (the product of the enantiomeric excess and the yield), its value decreases significantly if either of the constituent values are near zero. Accordingly, virtually no selectivity ($S < 0.05$) is observed between 100–120 bar and 180–200 bar due to the low values of the raffinate enantiomeric excess and raffinate yield, respectively. Between 130–170 bar, raffinate selectivity is moderate (around 0.25–0.35) and decreasing, due to the same trend being exhibited by the raffinate yields in this region.

Since similar sharp transitions have not been observed previously, further experiments were carried out to determine the underlying cause. Independent crystallization experiments have been conducted using pure enantiomers of cPA as the starting material instead of the racemate. Furthermore, the effect of the molar ratio was studied by decreasing the amount of cPA by half, yielding a molar ratio of 1. The results of the above mentioned experiments are summarized in Table 5. For comparison, col-
Figure 37: GAS resolution of cPA with (R)-PhEA. Effect of pressure on raffinate selectivity ($T = 45 \, ^\circ \text{C}$, $mr = 0.5$). For supplementary data, see Table A10 (Appendix C, p. A-9).

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>mr</td>
<td>0.5</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>$n_{\text{cPA}}$ [mmol]</td>
<td>0.62</td>
<td>0.62</td>
<td>0.31</td>
</tr>
<tr>
<td>$n_{\text{PhEA}}$ [mmol]</td>
<td>0.31</td>
<td>0.31</td>
<td>0.31</td>
</tr>
<tr>
<td>cPA configuration</td>
<td>racemic</td>
<td>(+)</td>
<td>(-)</td>
</tr>
<tr>
<td>$Y_r$ (100–120 bar)</td>
<td>0.40–0.65</td>
<td>0.40</td>
<td>0.47</td>
</tr>
<tr>
<td>$\hat{Y}_r$ (130–170 bar)</td>
<td>0.30–0.60</td>
<td>0.08–0.14</td>
<td>0.30–0.48</td>
</tr>
<tr>
<td>$\hat{Y}_r$ (180–200 bar)</td>
<td>&lt; 0.10</td>
<td>0.09</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Table 5: GAS resolution of cPA with (R)-PhEA. Summary of experimental conditions and raffinate yields ($Y_r$) of the independent crystallization experiments at $45 \, ^\circ \text{C}$, 2 ml MeOH, appr. 36 ml reactor volume.

Column A summarizes the experiments in which racemic cPA was used. Column B shows the results of experiments in which enantiopure cPA was used, column C shows the results of equimolar experiments ($mr = 1$). The raffinate yields have been categorized according to the three pressure regions between which sharp transitions have been observed.

The effects shown in Table 5 indicate a decomposition of the diastereomers in an equilibrium reaction: diastereomers form when an excess of cPA shifts the equilibrium away from decomposition. At the given volumetric cPA concentration, without excess cPA (as was the case for the equimolar experiments, see Table 5 column C), diastereomer formation does not proceed (raffinate $Y < 0.10$).
Increasing the pressure shifts the reaction towards decomposition, and for each salt there is a pressure value above which it becomes unstable: 120 bar for the \((+)-\text{cPA}-(R)-\text{PhEA}\) salt and 170 bar in the case of \((-)-\text{cPA}-(R)-\text{PhEA}\). The term "stable", in the context of these experiments, means that at the given volumetric \(\text{cPA concentration } c \left[ \text{g/dm}^3 \right] \), the diastereomeric salt does not undergo significant dissociation or re-dissolution. The term "significant" denotes the fact that although all diastereomers experience some dissociation/re-dissolution during the washing phase (as evidenced by the low yields), "stable" diastereomers are detectable gravimetrically (i.e. measurable amounts of diastereomer can be recovered from the reactor), chromatographically (i.e. yielding a well-defined peak in GC) and diffractographically (i.e. the corresponding peak pattern is clearly distinguishable in XRD). Conversely, the term "unstable" refers to diastereomers for which the dissociation (at the given pressure and concentration) proceeds to such a large extent that the mass of diastereomer falls below the gravimetric, chromatographic and diffractographic detection limits.

Between 100–120 bar, both salts are stable, but are affected by the equilibrium being shifted during the washing phase. Accordingly, of all the experiments, yields are the highest in this region, but remain below 0.65. Enantiomeric excesses, however, are almost zero, as both salts are produced in nearly equal amounts. Between 130–170 bar, only the \((-)-\text{cPA}-(R)-\text{PhEA}\) salt is stable, the \((+)-\text{cPA}-(R)-\text{PhEA}\) salt is above its stability threshold. Thus, yields are lower than those between 100–120 bar, as no \((+)-\text{cPA}-(R)-\text{PhEA}\) is recovered. However, due to the raffinate containing almost exclusively \((-)-\text{cPA}-(R)-\text{PhEA}\), enantiomeric excess in this region is excellent (\(> 0.8\)). Between 180–200 bar, both salts are in their unstable region. The \((-)-\text{cPA}-(R)-\text{PhEA}\) salt appears to be slightly more stable, as indicated by the ee values (0.2–0.4), however, the yields of both salts are below 0.1.

XRD analyses carried out on the raffinates support the findings of the independent crystallization experiments. Of particular interest are the raffinates obtained in the 100–120 bar region, an example of these (obtained at 110 bar) is compared against diastereomer standards (prepared from enantiopure \(\text{cPA}\) by vacuum evaporation) in Figure 38. As the relevant information in this diffractogram is carried by the relative – rather than absolute – peak heights (in addition to the peak positions), the series representing the GAS raffinate is shown shifted upwards along the vertical axis by 4000 units for clarity. The peaks of the GAS raffinate differ significantly from those of the diastereomer standards in both relative intensity and position. This latter difference is indicated by the marked peaks in Fig. 38. Peaks A and B denote a significant peak
on the diffractogram of $(-)$-cPA–($R$)-PhEA and $($+)−cPA–($R$)-PhEA, respectively, that are absent from the diffractogram of the 110 bar GAS raffinate. Incidentally, peaks A and B are also examples of the numerous peaks that appear on the diffractogram of one standard, but are absent from that of the antipode, furnishing evidence that – as expected – the two diastereomeric salts possess different crystalline structures. Peaks C1 and C2, conversely, are strong peaks on the diffractogram of the raffinate absent from those of the diastereomer standards. These differences indicate that the crystalline structure of the GAS raffinate is distinct from either diastereomer standard, i.e. between 100–120 bar, diastereomers crystallized by the GAS method are not the physical mixture of the $(-)$-cPA–($R$)-PhEA and $($+)−cPA–($R$)-PhEA salts. Similar analyses indicated that the raffinates formed in this pressure range contain neither racemic or enantiopure cPA nor the carbamate side product of ($R$)-PhEA.

Figure 39 shows the diffractogram of the same GAS raffinate as in Fig. 38, compared against the diffractogram of a diastereomer standard prepared from racemic cPA. The relative peak intensities and peak positions show a close correlation, thus the two materials can be assumed to have the same crystalline structure, i.e. between 100–120 bar, the GAS crystallization technique produces a racemic salt. Similar anal-
yses have confirmed that the crystalline structure of the raffinates formed between 130–170 bar matches that of the (−)-cPA–(R)-PhEA standard.

The raffinates produced by the GAS method were observed to have an unusual, very loose macroscopic structure, prompting their investigation by scanning electron microscopy. Figure 40 shows a SEM image of the raffinate of the GAS experiment carried out at 130 bar. The solid phase was composed almost entirely of irregular fibers, 500–700 nm in diameter and could reach up to 100 μm in length (somewhat similar to the structure of the IBU–(R)-PhEA GAS raffinates, see Fig. 26).

Based on the above results, the proposed reaction schemes for the different pressure ranges are presented in Figure 41.
Figure 40: GAS resolution of cPA with (R)-PhEA. Scanning electron microscope image of raffinate obtained at 130 bar, 45 °C.
Figure 41: Diastereomer formation reactions occurring during GAS resolution of cPA with (R)-PhEA for different pressure ranges, at $c = 3.61 \text{ g/dm}^3$ and $R$ between 11:1 and 19:1.
3.3.2. SAS Method

The resolution of racemic cis-permethric acid with (R)-(+-)1-phenylethanamine using methanol was the first system on which the SAS technique was attempted, and thus it was the system with which the semi-continuous crystallizer was tested. The goal of these experiments was to verify that crystallization occurs, thus the results presented focus on the raffinates. The effect of R was studied at 150 bar and 48 °C, the ratio achieved in the corresponding GAS experiment (R = 17.1:1) could not be achieved due to flow rate limitations.

In these experiments, 1500.0±0.5 mg (7.17±0.002 mmol) solid racemic cPA and 434.7±0.5 mg (3.59±0.004 mmol, mr = 0.5) liquid (R)-PhEA was dissolved in 7.5 ml MeOH and injected into the crystallizer according to the procedure described in 2.4.2. The crystallizer was maintained at 150 bar and 48 °C, the separator was maintained at 40 bar and 40 °C. Raffinate yields were characterized by \( \bar{Y} \). Different values of R were set by keeping the flow rate of scCO\(_2\) constant and varying the methanol solution flow rate between 3.1 ml/min (R = 14.7:1) and 4 ml/min (R = 11.4:1).

Figure 42 shows ee values in the raffinates. In the range investigated, ee values are not discernibly affected by R, remaining between 0.8–1.0 (–)-cPA. Of particular interest are the points at 13.0:1, where the diastereomers have optical purities of \( \text{ee}(–) = 0.954 \) and \( \text{ee}(–) = 0.925 \) (indicating (–)-cPA purities of 97.7% and 96.3%, respectively). The ee values are comparable to those obtained in with the GAS method.

![Figure 42: SAS resolution of cPA with (R)-PhEA. Optical purities of the raffinates at 150 bar, 48 °C. Note overlapping points at 12.0:1. For supplementary data, see Table A11 (Appendix C, p. A-9).](image-url)
both techniques yielding exceptionally high purity diastereomers in a single step.

The yields of raffinates are shown in Figure 43. The trend is closely linear with respect to $R$, increasing steadily from 0.069 to 0.253 (at $R = 14.7:1$). The GAS experiment conducted at the same pressure (150 bar, albeit at 45 °C instead of 48 °C), produced a raffinate yield of 0.315 at $R = 17.1:1$, which fits well into this trend.

Figure 44 shows a SEM image of the raffinate obtained at $R = 14.7:1$. Its structure is highly similar to that of the GAS raffinates: high length-to-diameter ratio fibers, with a narrow diameter distribution (400–700 nm) and typical lengths exceeding 100 µm. This structure resulted in an unusually low bulk density for both GAS and SAS raffinates. Whereas cPA–(R)-PhEA standards prepared by vacuum evaporation (the method used for the in vacuo experiments, see Section 2.3.2) had an estimated bulk density of 100 kg/m³, diastereomers crystallized by the antisolvent methods had bulk densities of approximately 5 kg/m³. Figure 45 shows a photograph of a (−)-cPA–(R)-PhEA standard compared against the SAS raffinate obtained at $R = 14.7:1$. 

Figure 43: SAS resolution of cPA with (R)-PhEA. Yields of the raffinates at 150 bar, 48 °C. For supplementary data, see Table A11 (Appendix C, p. A-9).
Figure 44: SAS resolution of cPA with (R)-PhEA. Scanning electron microscope image of raffinate obtained at 150 bar, 48 °C, $R = 14.7:1$.

Figure 45: SAS resolution of cPA with (R)-PhEA. Left: 100 mg of a (−)-cPA–(R)-PhEA standard prepared by vacuum evaporation, bulk density $\sim 100 \text{ kg/m}^3$. Right: 100 mg of SAS raffinate obtained at 150 bar, 45 °C, $mr = 0.5$, $R = 16.9:1$, bulk density $\sim 5 \text{ kg/m}^3$. 


3.4. DISCUSSION

3.4.1. EFFECT OF RACEMATE–RESOLVING AGENT INTERACTION

The most important conclusion that can be drawn from the results presented above is that the effects of factors investigated for more than one system (reaction time, pressure, temperature, antisolvent:solvent ratio) adequately describe only resolution system in which they have been measured. Although some generalized trends for certain factors are established below, the racemate–resolving agent pairing still plays the largest role in determining the eventual outcome of experiments. This is likely due to the complexity of the resolution systems: the reactions leading to chiral discrimination must proceed in a ternary (\textit{in situ} method, racemate–resolving agent–CO$_2$) or quaternary (antisolvent methods, racemate–resolving agent–solvent–CO$_2$) solution phase in equilibrium with a binary solid phase (racemate–resolving agent diastereomeric salt). Due to the use of scCO$_2$, properties of the system can vary significantly as a function of pressure or temperature.

The importance of choosing an appropriate resolving agent is best illustrated by the findings [183] that PhEA forms a carbamate type self-derivative compound with carbon dioxide, as shown in Figure 46. XRD analyses carried out on raffinates obtained in the \textit{in situ} resolution of IBU with (\textit{R})-PhEA have confirmed that the carbamate is present along with the \textit{(R)}-\textit{(-)}-ibuprofen–\textit{(R)}-\textit{(+)}-1-phenylethanamine salt. Figure 47 shows that the superposition of the carbamate and diastereomeric salt diffractograms adequately describes the peaks of an \textit{in situ} raffinate, therefore, the raffinate contains a physical mixture of (PhEA)$_2$CO$_2$ and \textit{(R)}-IBU–\textit{(R)}-PhEA.

![Figure 46: Reaction of PhEA with carbon dioxide to form 1-phenylethananminium (1-phenylethyl)carbamate](image)

Despite the byproduct formation, PhEA was used for the IBU–PhEA \textit{in situ} experiments, because the formation of the diastereomeric salts is thermodynamically favoured over the kinetically controlled carbamate formation. Figure 48 shows that the carbamate content of IBU–\textit{(R)}-PhEA \textit{in situ} raffinates increases until at least 3.0 h
Figure 47: *In situ* resolution of IBU with (R)-PhEA. Diffractogram of raffinate obtained at 150 bar, 40 °C and 3.0 h reaction time (red, shifted upwards 800 units), compared against the diffractogram of the self-derivative carbamate salt of 1-phenylethanolamine (dark blue) and the diastereomeric salt of (R)-IBU with (R)-PhEA (light blue, simulated from single crystal data). Note the peak around 9°, which is contributed by the carbamate only.

Figure 48: *In situ* resolution of IBU with (R)-PhEA. Diffractograms of raffinates obtained at different reaction times at 150 bar and 40 °C. Dashed line indicates the characteristic peak of the carbamate derivative.
reaction time, but cannot be detected for reaction times over 21.0 h. Note that this conclusion – as noted earlier – only applies to the IBU–PhEA system: the carbamate byproduct could not be detected in the raffinates of cPA–(R)-PhEA experiments, and reaction time did not exert a significant effect on the resolution (see Section 3.2.1, p. 76).

Fig. 48 also shows that no significant amount of carbamate is present in the raffinate after 1.3 h reaction time, thus PhEA was also a suitable resolving agent for the antisolvent techniques, as the diastereomer formation in these methods occurs on the order of minutes. Both the GAS and SAS methods could be realized using (R)-PhEA (and, for certain experiments with IBU, (S)-PhEA) as the resolving agent. This further highlights the importance of racemate–resolving agent pairing: applied to the same racemates, the antisolvent processes were unsuccessful using (S)-BAB for either racemate. Additionally, no resolution whatsoever could be achieved for the IBU–(S)-BAB system (hence the omission of this system from the results).

3.4.2. Effect of Time

The effect of time on the in situ technique was investigated for the IBU–(R)-PhEA and cPA–(S)-BAB systems. Although both the racemate and resolving agent was different, the difference in the effect is still remarkable. While the reaction between cPA and BAB appears to proceed to a large extent within 1 h (as indicated by the extract enantiomeric excess, see Section 3.2.1 Fig. 33 on p. 73), at 150 or 200 bar the IBU–(R)-PhEA system does not reach equilibrium even after 72 h (see Section 3.1.1, Fig. 19 on p. 55).

Differences in the effects of reaction time in in situ experiments mostly stem from differences resolving agents, as the experiments were designed such that the racemates would be completely soluble in the amount of scCO₂ used. Experiments conducted on the IBU–(R)-PhEA system showed significant dependence on reaction time (see Fig. 19 on p. 55), presumably due to the competitive PhEA byproduct formation detailed in Section 3.4.1. In contrast, experiments carried out with (S)-BAB (which has a small but measurable solubility in scCO₂) showed that an initial equilibrium establishes rapidly in < 1 h reaction time, with a much less pronounced effect thereafter.

For in vacuo experiments, the effect of reaction time depends on whether a difference exists between the equilibrium composition that establishes during vacuum evaporation and that which forms in carbon dioxide. No such difference exists for
the cPA–(R)-PhEA system, resulting in constant extract ee values for reaction times between 1–50 h (see Section 3.2.1 p. 76). On the other hand, the significant effect of reaction time for the IBU–(R)-PhEA system (in the analogous packed column extraction experiments, see Section 3.1 on p. 54) can be assumed to stem from different equilibrium states.

The effect of reaction time was not investigated for the antisolvent techniques, since view cell experiments confirmed that crystallization in these systems occurs on the order of minutes. Nonetheless, batch antisolvent experiments were still ran for approximately 1 h in order to ensure complete precipitation. Furthermore, it must be noted that the average residence time for SAS experiments was approximately 2 min, which is commensurable with the crystallization time observed visually in the view cell. This could explain the lower yields in SAS experiments (compared to GAS experiments under similar conditions), as it is possible that the average residence time limits the extent to which crystallization proceeds.

### 3.4.3. Effect of Pressure and Temperature

The effect of pressure is markedly different not only between resolution systems but resolution techniques as well. For in situ resolutions, higher pressures appear to be favourable for both the IBU–(R)-PhEA and cPA–(S)-BAB systems, albeit the interaction with reaction time is significant. At 1 h, the effect of pressure is almost negligible for the IBU–(R)-PhEA system ($\hat{F}$ increases from 0.2 to 0.25, see Section 3.1.1, Fig. 19 on p. 55), while decreasing the pressure from 200 bar to 150 bar virtually eliminates chiral discrimination in the cPA–(S)-BAB system (extract ee decreases from almost 0.8 (+)-cPA to essentially 0, see Section 3.2.1 Fig. 34 on p. 73). For the GAS technique, the differences are even more apparent: despite using the same resolving agent, pressure exerts no significant effect on the IBU–(R)-PhEA system between 100–200 bar (see Section 3.1.2 Fig. 22 on p. 58), while the behaviour of the cPA–(R)-PhEA system undergoes two rapid changes in the same pressure range, producing diastereomers ranging from nearly racemic to nearly enantiopure ($ee_{(-)} = 0.021$ at 120 bar versus $ee_{(-)} = 0.940$ at 150 bar, see Section 3.3.1 Fig. 36 on p. 77).

The effect of temperature, which was investigated for the two in situ systems (IBU–(R)-PhEA, cPA–(S)-BAB), again displays a similar generic trend: higher temperatures appear to be favoured (as expected from the Arrhenius equation). However, while increasing the temperature to 55 °C only causes the cPA–(S)-BAB system to produce diastereomers of a markedly lower ee ($ee_{(-)} = 0.221$ versus $ee_{(-)} = 0.607$)
at 45 °C, see Section 3.2.1 Table 4), the IBU–(R)-PhEA system fails to yield any solid diastereomers at temperatures over 50 °C (see Section 3.1.1).

Due to the strong dependence of pressure and temperature effects on the racemate–resolving agent pairing, a detailed investigation of these effects is indispensable for in vacuo, in situ as well as antisolvent experiments.

3.4.4. Effect of solvent composition

The effect of the antisolvent:solvent ratio, either defined as the mass ratio \( R \) (see Eq. 2.1 on p. 38), mole ratio \( R_m \) (see Eq. 2.2 on p. 38, not to be confused with the molar ratio \( m_r \)) or the mass flow rate ratio \( R \) (see Eq. 2.3 on p. 43) is the only factor in which there appear to be broad similarities between two systems. Investigated for the IBU–(R)-PhEA GAS and cPA–(R)-PhEA SAS systems, it appears to exert almost no effect on raffinate enantiomeric excesses (see Section 3.1.2 Fig. 25 and Section 3.3.2 Fig. 42), while increasing raffinate yields at higher values (see Fig. 23 on p. 60 and Fig. 43 on p. 85). For the IBU–(R)-PhEA system, yields level off at higher ratios after the initial linear increase, according to a saturation curve. This behaviour is observed when using either methanol or ethanol, with the two curves showing a high degree of similarity when plotted against the molar \( \text{CO}_2: \text{solvent} \) ratio defined by Eq. 2.2 (see Section 3.1.2 Fig. 24 on p. 60). When using methanol, the yields in the IBU–(R)-PhEA and cPA–(R)-PhEA systems also show similar trends, this is shown in Figure 49.

![Figure 49: Antisolvent resolutions of IBU and cPA with (R)-PhEA. Effects of the CO₂:methanol mass ratio (GAS) and mass flowrate ratio (SAS) on the raffinate yields. For supplementary data, see Tables A4 and A11 (Appendix C, p. A-6 and A-9).](image-url)
Since enantiomeric excesses are not affected by the solvent composition (as shown in Fig. 25 on p. 61 and Fig. 42 on p. 84), the solvent ratio can be optimized with respect to the yields. Increasing the CO\textsubscript{2}:solvent ratio increases the raffinate yields, however, this increase is limited by the saturation curve. Furthermore, the ratios are limited in the GAS technique by the fact that increasing the ratio is done by decreasing the amount of organic solvent, which – due to the solubility limit of the racemate and resolving agent in the organic solvent – decreases the amount of materials processed in one batch. Decreasing the CO\textsubscript{2}:solvent ratio decreases the yields, but as there is more organic solvent, more material can be processed in a single batch. Similar considerations apply to the SAS technique, although in that case the limitation stems from the capacity of the crystallizer and separator vessels, rather than the amount of initial solvent. However, at higher values of \( R \), the specific consumption of CO\textsubscript{2} increases, i.e. more CO\textsubscript{2} is required to produce a given amount of diastereomer.

### 3.4.5. Method development for antisolvent processes

Although, as discussed in the previous section, the behaviour of the antisolvent resolution methods differs significantly, their development and implementation comprised largely similar steps. These have been generalized and compiled here into a generic template for screening and developing antisolvent resolution processes. For the purposes of this section, assume that a resolution system is proven to achieve chiral discrimination in supercritical carbon dioxide (e.g. using the \textit{in situ} or \textit{in vacuo} methods).

First, one or more suitable solvent or solvent combination (henceforth referred to as "solvent") must be selected, based on solvent power and compatibility with carbon dioxide. The solvent must be able to efficiently dissolve not only the racemate and the resolving agent, but the formed diastereomers as well. A large number of resolution systems are based on acid–base interaction, thus the diastereomers in these systems are salts, the ionic character of which requires solvents of appreciable polarity (ionic salts, however, are not a prerequisite of successful resolution using scCO\textsubscript{2}: racemic \textit{trans}-1,2-cyclohexanediol could be resolved using L-tartaric acid via intermolecular complex formation [84, 85, 188]). However, even if the diastereomers are ionic, highly polar solvents are typically unsuitable for the antisolvent resolution due to their incompatibility (in this case, immiscibility) with carbon dioxide. Solvents must be stable under pressure and unreactive towards but miscible with carbon dioxide. Solvent selection in terms of solvent power can be done in simple atmospheric solu-
bility tests, suitable candidates can be tested for compatibility with carbon dioxide in view cell experiments or based on literature data.

In addition to determining solvent suitability, view cell experiments (described in Section 2.2.3) are valuable in establishing the range of process parameters (pressure, temperature, racemate/resolving agent concentration in the solvent and CO$_2$:solvent ratio) in which the resolution is possible. The ability to visually observe the reaction mixture, as well as the variable volume of the view cell (allowing the alteration of pressure without affecting the composition of the reaction mixture), allow the efficient screening of different parameter combinations. However, due to the design of the equipment, efficient separation of the precipitates from the unreacted components is not possible. Therefore whether chiral discrimination occurs can only be inferred from the appearance of a precipitate and by measuring the enantiomeric excess of CO$_2$ phase by sampling. Despite this limitation, view cell experiments can identify suitable solvents and process parameter combinations that can be used as starting points for GAS experiments.

Suitable systems (in the sense of racemate–resolving agent–solvent groups) are transferred to the batch reactor. The main advantage of the GAS technique over the view cell is its ability to achieve separation of CO$_2$-soluble compounds from precipitates. Since separate extract and raffinate phases are produced, yields and enantiomeric excesses can be determined, allowing for precise characterization of a given experiment. Furthermore, the separated fractions can be studied via powder X-ray diffraction or scanning electron microscopy, though the latter is typically only used for the raffinate (since the extract is collected in a liquid trap and evaporated in vacuum, rendering its microscopic structure unrepresentative). The GAS method can be used to carry out detailed studies into the effects of process parameters (chiefly, pressure, temperature, CO$_2$:solvent ratio and reaction time) and their interactions upon yields, optical purities and diastereomer structure.

Once a combination or range of optimal process parameters have been identified, the resolution can be implemented using the SAS technique. Although the experiments presented in Sections 3.1.3 and 3.3.2 were only preliminary, they indicate that if the process parameters of a successful GAS experiment are applied to the SAS method (with $R$ being a direct substitute for $R$), similar results (in terms of optical purities and raffinate structure) can be achieved. The SAS technique has the added benefits of larger capacity (approximately one order of magnitude with respect to the mass of racemate) and more tightly controlled experimental conditions: while the
racemate and resolving agent are exposed to CO$_2$ in the entire range of pressures from atmospheric up to the desired process pressure (and view cell measurements suggest that precipitation may begin before the desired pressure is reached), the solution in the SAS technique comes into contact with a stream of scCO$_2$ maintained at the desired pressure (typically to within ±5 bar).
CONCLUSION

Three resolution systems have been investigated: the resolution of ibuprofen with resolving agent 1-phenylethanamine (using (R)-(+)-1-phenylethanamine in most experiments, (S)-(−)-1-phenylethanamine in some additional studies), and the resolution of cis-permethric acid with the resolving agents (R)-(+)1-phenylethanamine and (S)-(+)2-(N-benzylamino)butan-1-ol.

The novel, solvent-free resolution in a heterogeneous-phase reaction with supercritical CO\(_2\) (the in situ method) was demonstrated on the ibuprofen–(R)-(+)1-phenylethanamine system. At 200 bar and 40 °C, both the (R)-(−)-ibuprofen-enriched diastereomers and the (S)-(+)ibuprofen-enriched unreacted enantiomers had enantiomeric excesses of approximately 0.6, with an overall recovery of 83% (with respect to the racemate and the resolving agent). By raising the temperature to 50 °C, unreacted enantiomeric mixtures of nearly ee = 0.8 could be obtained (though the resolution was unsuccessful at higher temperatures). Although no organic solvent and virtually no downstream processing of products was required to obtain the aforementioned optical purities in a single step, it must be noted that the results required reaction times in excess of 80 h.

Techniques using supercritical CO\(_2\) as an antisolvent were also successfully applied to the ibuprofen–(R)-(+)1-phenylethanamine system. The effect of process parameters was investigated in detail. At 100 bar, 45 °C, with half-equivalent amounts of resolving agent, using methanol with an antisolvent:solvent mass ratio of 11.3:1 g/g and a volumetric IBU concentration of 4.17 g/dm\(^3\) CO\(_2\), the optical purity in the diastereomeric salts and unreacted enantiomers was 0.747 and 0.589, respectively, with an overall recovery of 65%. Increasing the antisolvent:solvent ratio increased diastereomer yields according to a saturation curve while having negligible effect on their optical purities, only marginally influenced by the choice of solvent. Although the antisolvent:solvent ratio did not influence the crystalline structure (as determined by powder X-ray diffraction), scanning electron microscopy revealed significant differences between crystal habits of diastereomers obtained at different ratios. The resolution could be carried out at or above equimolar quantities of resolving agent, attributed to the decomposition of the diastereomers. This enabled a two-step resolution, in which diastereomers formed in an antisolvent experiment were subjected to a second resolution step with no further resolving agent added. The second step yielded practically racemic unreacted enantiomers and diastereomers with an enantiomeric
excess of 0.9 \((R)-(\text{-})\)-ibuprofen, which exceeds the optical purity at the eutectic point of ibuprofen enantiomers. While the solvent consumption of this facile two-step process is remarkably low compared to traditional crystallizations from organic solvents, yields were significantly lower due to losses resulting from the laboratory-scale implementation.

A published resolution method [107] for ibuprofen, based on crystallization from organic solvent using \((S)-(\text{-})\)-phenylglycinol as a resolving agent, yielded a de of 53% with yields around 50%. The in situ method produced diastereomers with slightly higher yields and optical purity \((\hat{Y} = 0.56, \text{ee}_{(R)} = 0.58\) at 200 bar and 40 °C), although only after 73.5 h. The GAS method (at 150 bar, 45 °C, \(R = 29.5\)) produced slightly lower yield with higher optical purity in the raffinate \((Y = 0.43, \text{ee}_{(R)} = 0.78\), however, the required operation time was around 3 h. The two-step purification at 130 bar, 45 °C, using an initial SAS resolution with \(mr = 1\) and subjecting the raffinate to a GAS resolution with no additional resolving agent, diastereomers with an overall \(Y = 0.08\) (albeit using only 22.5% of the raffinate) and \(\text{ee}_{(R)} = 0.90\) could be obtained. In conclusion, the in situ method yielded results comparable to published methods without the use of organic solvents, after much longer reaction times. The antisolvent methods produced higher purity diastereomers with operation times on the order of a few hours, albeit in lower yields.

The in situ method was successfully applied to the resolution of \(\text{cis}\)-permethric acid using \((S)-(\text{+})\)-2-\((N\)-benzylamino\)butan-1-ol. The effects of process parameters were investigated in detail. In contrast with the resolution of ibuprofen, the reaction time was found to have a minor effect, as good results could be obtained even after 1 h. At 200 bar and 45 °C, diastereomers with an enantiomeric excess of 0.781 \((1S,3S)-(\text{-})\)-\(\text{cis}\)-permethric acid could be obtained at 60% theoretical yield (based on the total amount of diastereomer assuming a complete, irreversible diastereomer crystallization). A study of temperature effects suggests that these results could be further improved by lowering the temperature to 35 °C. Although the yields and optical purities are inferior to those obtained in published resolution methods, they were achieved in a single step without the use of organic solvents and with virtually no downstream processing required (compared to the multiple recrystallizations from organic solvents in published methods).

The resolution of \(\text{cis}\)-permethric acid with \((S)-(\text{+})\)-2-\((N\)-benzylamino\)butan-1-ol from organic solutions using pH-driven crystallizations [142] reported the obtained diastereomeric salts as "optically pure", however, 50% of the initial \(\text{cis}\)-permethric acid
remains at the end of the resolution. At 200 bar and 45 °C, recovery (with respect to the complete amount of material measured in) for the in situ system is 0.77, however, the fractions are not optically pure (extract ee(+) = 0.28, raffinate ee(−) = 0.51).

Using (R)-(+) -1-phenylethanamine, the antisolvent resolution of cis-permethric acid was successful. The effect of pressure on optical purities showed unusual, sharp transitions, initially attributed solely to diastereomer decomposition. Further investigation revealed that between 100–120 bar, a stable racemic salt forms, while between 130–200 bar, the optical purity of the crystalline phase is influenced by the relative stability of the individual diastereomers. Between 130–170 bar, where only the (1S,3S)-(−)-cis-permethric acid salt is stable, diastereomers with ≥ 0.8 (up to 0.94) ee (1S,3S)-(−)-cis-permethric acid could be obtained. An apparatus for semi-continuous antisolvent resolution was developed and successfully tested. Although the diastereomer optical purities exceed previously published results, yields are diminished due to diastereomer decomposition.

A crystallization-based resolution of cis-permethric acid, using amines derived from natural carenes [143], produced both enantiomers of cis-permethric acid in 82–86% yield with optical purities reported as 95% for (1R,3R)-(+) -cis-permethric acid and ≥ 98% for (1S,3S)-(−)-cis-permethric acid. However, these results were obtained using several recrystallizations (twice and three times for the (−) and (+) enantiomers, respectively), with one recrystallization for (1R,3R)-(+) -cis-permethric acid involving benzene. In contrast, the GAS method at 150 bar and 45 °C yielded the (1S,3S)-(−)-cis-permethric acid–(R)-(+) -1-phenylethanamine salt in 94% purity in a single step using methanol. Due to diastereomer dissociation, however, the extract optical purity was low. The SAS method produced diastereomer salts with 0.93 ee and Ŷ = 0.15, extract optical purity was 0.32 with Ŷ = 0.09. In conclusion, the antisolvent methods yielded optical purities comparable to traditional methods with reduced solvent use and operation times, albeit with lower yields.

Based on the investigations carried out, some general guidelines for developing antisolvent resolution techniques have been presented.
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112
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1. Introduction

The preparation of optically active compounds in enantiopure form (either by resolution or asymmetric synthesis) is a key issue in the chemical industry, particularly in the food and pharmaceutical sectors. In the latter especially, the high number of chiral compounds produced in large scale requires that their syntheses be both economically and environmentally favorable. For our experiments, we chose ibuprofen (IBU) as a model compound, a chiral drug often marketed in racemic form, even though only (S)-IBU is biologically active [1]. Despite (R)-IBU undergoing bioinversion in the body [2], (S)-IBU is 100 times more effective when administered in enantiopure form [3].

In this paper, we report a resolution technique based on diastereomeric salt formation according to the modified Pope–Peachey method. The original resolution method [4] developed by Pope and Peachey involved adding the resolving agent and an achiral auxiliary to the racemic mixture, each in half-equivalent quantities. The resolving agent forms a compound—typically a salt—preferentially with one of the enantiomers and precipitation. The achiral auxiliaries increase the solubility of the unreacted enantiomer mixture, thus the precipitated compound can be removed by simple filtration. The modified method omits the achiral auxiliary, relying on the solubility difference between the enantiomer-resolving agent compound and the unreacted enantiomers.

For our experiments, we chose (R)-1-phenylethylamine (PhEA) as the resolving agent. The IBU–PhEA resolution system was first described by Fogassy et al. [5] using supercritical fluid extraction (SFE): (±)-IBU and (R)-PhEA were separately dissolved in organic solvent, then united. The solvent was evaporated, the (R)-IBU–(R)-PhEA salt crystallizing preferentially, yielding a solid mixture of (R)-IBU-rich salt and (S)-IBU-rich enantiomeric mixture. This solid material was extracted with supercritical carbon dioxide (scCO₂).

Due to the polar nature of the (R)-IBU–(R)-PhEA salt, it is principally insoluble in carbon dioxide, and the (S)-IBU-rich enantiomeric mixture was extracted by the highly apolar scCO₂. Further investigation by Molnár [6] showed that for IBU–PhEA salts prepared under identical conditions in organic solvent, the pressure of the extraction influences the optical purity of the extract and raffinate. This suggests that salt decomposition and formation takes place in the supercritical phase, with an equilibrium different from that attainable under atmospheric conditions. The aim of our work was to investigate the kinetics of this reaction by eliminating the organic solvent, reacting the IBU and PhEA in pure supercritical carbon dioxide.

2. Materials and methods

Racemic ibuprofen (≥98%, GC) was purchased from Sigma–Aldrich Ltd. (Budapest, Hungary). (R)-1-phenylethylamine (≥99%, GC) was purchased from Merck Ltd. (Budapest, Hungary). Carbon dioxide (99.5%) was purchased from Linde Gas Hungary Co. Ltd (Budapest, Hungary). Methanol (≥99.9%, GC) used for liquid
trapping and GC analysis was performed from Sigma–Aldrich Ltd. (Budapest, Hungary).

The extraction was performed in two basic steps: formation of the (R)-IBU–(R)-PhEA salt in a batch reactor, and the separation of the salt and unreacted IBU by flowing scCO₂ through the reactor vessel, effectively operating it as an unsteady-state continuous stirred tank reactor (CSTE). The high-pressure reactor, manufactured by the Research Institute of Applied Chemistry at the University of Miskolc, has a useful volume of 30.6 ml. The experimental apparatus is shown in Fig. 1. Carbon dioxide is fed by an Isco 2600 syringe pump (1) through an inlet valve (2) and into the stainless-steel reactor vessel (3). The reactor is equipped with a pressure transducer (4), a thermocouple (5) and a rupture disk calibrated to 250 bars (not pictured). Adequate mixing was ensured by a stir bar (6) rotated by a magnetic stirrer (7). The reactor was maintained at a constant temperature via thermostated water (8a, 8b) flowing through the reactor’s aluminum jacket, circulated by a thermostat (not pictured). Carbon dioxide leaving the reactor passed through a filter (9) and an outlet valve (10) where it expanded to atmospheric pressure. The particles that precipitated during the pressure drop were collected in a liquid trap (11).

The relative amounts of PhEA and IBU are described by the molar ratio (mr):

\[
mr = \frac{m_{\text{PhEA}}}{m_{\text{IBU}}} \quad (1)
\]

In accordance with the modified Poppe–Peache method, most experiments were performed with mr = 0.5, which Molnar [7] also found to be the optimal value. In some experiments, the reaction progress was tracked by measuring the enantiomeric excess of IBU in the scCO₂ phase at certain time intervals. To this end, samples were taken by flowing a small amount of scCO₂ through the reactor. However, compared to IBU, PhEA is removed from the reactor only in negligible quantities (as confirmed by analytical measurements), thus with each sampling mr increases. Since Molnar found that the effect of mr is negligible in the range of 0.45–0.65 [7], sampled experiments were started with mr = 0.45 and the samplings were limited to ensure that mr does not increase beyond 0.65.

The first stage in each experiment was measuring IBU and PhEA into the reactor. In experiments without sampling mr = 0.5, 0.2000 ± 0.0020 g (0.97 mmol) IBU and 0.0587 ± 0.0020 g (0.49 mmol) PhEA were measured. In measured experiments, \(mr = 0.45\), 0.2000 ± 0.0020 g (0.97 mmol) IBU and 0.0529 ± 0.0020 g (0.44 mmol) PhEA were used. After measuring in the materials, the reactor was sealed, thermostatted to the desired temperature and filled with carbon dioxide. Once the desired pressure was reached, the reaction was started by activating the stirrer. Samples were taken by passing 2 ml scCO₂ through the reactor (measured by the syringe pump at the reaction pressure and temperature), no more than four times in a given reaction (in order to maintain mr below 0.65). After the desired reaction time elapsed, 60 ml scCO₂ (measured by the syringe pump at the reaction pressure and temperature) was flowed through the reactor to wash out the (S)-IBU-rich scCO₂ phase (referred to as the extract). During both sampling and washing, temperature (via the thermostatted water in the reactor jacket) and pressure (via the syringe pump controller) were kept constant. Upon completion of the washing, stirring was stopped, the reactor was depressurized and the (R)-IBU–(R)-PhEA salt (referred to as the raffinate) was recovered in solid form.

The optical purity of IBU (in both the extract and raffinate) was measured by gas chromatography using a Finnigan THERMO GC fitted with a SUPELCO β-DEX™ 120 column (stationary phase containing 20% permethylated β-cyclodextrin, length 30 m, ID 0.25 mm, film thickness 0.25 μm). Samples were prepared in methanol in concentrations of 1–5 mg/ml. 0.6 μl was injected onto the column. The temperature program was 100 °C for 2 min. ramp 20 °C/min, 160 °C for 8 min, ramp 10 °C/min, 210 °C for 8 min. ramp 10 °C/min, 230 °C for 4 min. Helium was used as carrier (140 kPa, 50 ml/min split flow), detection was done via FID at 250 °C. The retention times of (S)-IBU and (R)-IBU were 20.2 and 20.5 min, respectively.

The optical purity was described by the enantiomeric excess, defined as:

\[
e e = \frac{S - R}{S + R} \quad (2)
\]

Here, S and R signify the peak areas of (S)-IBU and (R)-IBU, respectively, on the chromatogram. Values of ee range from −1 (pure (R)-IBU) to +1 (pure (S)-IBU).

The yield of the extract and raffinate were defined to account for the incomplete removal of the dissolved component. Washing an optimal CSTE of volume \(V_r\) with \(V_w\) volume of solvent, the ratio of extracted mass (m) to the initial mass (m₀) is given by the following equation:

\[
m = \frac{m}{m_0} = 1 - e^{-(V_w/V_r)} \quad (3)
\]

The extract yield was calculated by first defining the theoretical extract mass at full conversion (\(m_e\)) from the mass of IBU measured into the reactor (\(m_{\text{IBU}}\)) by assuming ideal mixing (confirmed by experiments to be reasonable) and substituting the measured volume of scCO₂ (\(V_r\)) that flowed through the reactor. \(m_e\) was calculated from the volume change in the jacketed, thermostatted piston of the pump, accounting—where necessary—for the difference in densities arising from the temperature difference between the reactor and the pump.

\[
m_e = \frac{m_{\text{IBU}}}{2} \left(1 - e^{-(V_w/30.6\text{ml})}\right) \quad (4)
\]

The formation of the diastereomeric salt changes the reactor volume by less than 0.5%, and thus it can be neglected.

The extract yield (\(Y_e\)) is calculated from the mass recovered in the extract (\(m_e\)) by:

\[
Y_e = \frac{m_e}{m_r} \quad (5)
\]

The yield of the raffinate was calculated to account for the part of IBU that was not extracted and the PhEA which can be assumed to remain entirely within the reactor (analytical measurements showed this assumption to be reasonably correct). A theoretical raffinate mass was calculated for full conversion from the masses of IBU and PhEA measured into the reactor (\(m_{\text{IBU}}\) and \(m_{\text{PhEA}}\), respectively) and the theoretical mass of the extract (\(m_e\)):

\[
m_r = m_{\text{IBU}} - m_e + m_{\text{PhEA}} \quad (6)
\]
The raffinate yield ($Y_r$) was calculated from this theoretical mass and the actual recovered raffinate mass ($M_r$) by:

$$Y_r = \frac{m_r}{m_t}$$  \hspace{1cm} (7)

The yields and optical purities can be used to calculate the dimensionless $F$ parameter which describes the overall efficiency of the resolution. The original equation [6] for the $F$ parameter is:

$$F = e e_r - Y_r + e r - Y_t$$  \hspace{1cm} (8)

Here, $Y$ and $ee$ denote the yield and enantiomeric excess, respectively, the indices $e$ and $r$ refer to the extract and raffinate, respectively. In this equation, yields are calculated relative to the entire racemic mass, thus they are in the range 0–0.5. However, we calculated yields relative to a theoretical maximum, thus our yield values fall between 0 and 1. Furthermore, enantiomeric excesses used in this equation must be absolute, i.e. in the range of 0–1 irrespective of which enantiomer is in excess, whereas our definition results in values between −1 and 1. According to the original definition, the $F$ parameter takes on values between 0 and 1. In order to obtain comparable values using our values for $ee$ and $Y$ calculated with the methods above, the equation needs to be modified:

$$F = \frac{|ee| \cdot Y_e + |ee| \cdot Y_t}{2}$$  \hspace{1cm} (9)

3. Results and discussion

The effects of pressure ($p$; 100, 150 and 200 bars) and reaction time (t; 1–120 h) were studied by performing 8 experiments of different durations at each pressure ($n=0.5$, no sampling). At 100 bars, the solubility of ibuprofen was found [8] to decrease rapidly above 40 °C, therefore all experiments were performed at 40 °C. The $F$ parameter was computed for each experiment and the $F(p, t)$ relationship was described by a fitted quadratic surface:

$$F = -0.16 + 5.3 \times 10^{-3} p - 5.5 \times 10^{-3} t - 1.56 \times 10^{-5} p^2 + 4.98 \times 10^{-7} pt + 4.86 \times 10^{-6} t^2$$

This surface is shown in Fig. 2 as contour lines with a spacing of 0.02, along with the measured data points. It is clearly visible that the $F$ parameter increases with longer reaction times at 150 and 200 bars, but remains nearly constant at 100 bars. An alternate but equivalent description is that at shorter reaction times pressure exerts a small effect on the $F$ parameter, but for reactions longer than 48 h, higher pressures yield much higher $F$ parameter values. The behavior of the system can be explained by three factors.

First, the markedly different behavior at 100 bars indicates an equilibrium reaction: while at 150 or 200 bars, the equilibrium was not attained even after 120 h (hence the constantly increasing values of $F$), at 100 bars the equilibrium was reached in under 1 h (the length of the shortest experiment) and remained constant thereafter. As can be inferred from Fig. 2, the equilibrium value of the $F$ parameter at 100 bars is significantly lower than at 150 or 200 bars (the equilibrium value of these latter two pressures cannot be determined from the data, as equilibrium was not reached).

Second, the values of $F$ appear to be increasing slightly faster at 200 bars than at 150 bars, indicating that the reaction rate increases with increasing pressure. This can be explained by the diastereomers salt formation reaction having negative activation volume and thus being favorable at higher pressures. As mentioned above, at 100 bars the time to reach equilibrium was shorter than the time of the shortest experiment, and thus the experiments furnish no data regarding the reaction rate. However, given the low equilibrium value of the $F$ parameter, it can be assumed that the reaction rate at 100 bars is lower than at higher pressures.

Third, similar to the results of Keszei [6], we have observed an increase in optical purity with increasing pressure. This could be due to the differing unit cells of the diastereomeric salts: the $(R)$-IBU-$(R)$-PhEA and $(S)$-IBU-$(R)$-PhEA salts have unit cell volumes of 1997.2 Å$^3$ and 2005.2 Å$^3$, respectively [9]. Although small, this difference suggests that as pressure increases, formation of the $(R)$-IBU-$(R)$-PhEA salt would become favorable, which is exactly what we observed.

The effect of temperature on the optical purity of IBU in the carbon dioxide phase was studied in three sampled experiments at 40 °C and compared to one sampled experiment carried out at 50 °C. The results are shown in Fig. 3.

Both data series appear to confirm that the diastereomer formation is an equilibrium reaction: the optical purity increases rapidly in the first 24 h, gradually nearing an equilibrium over 3–5 days. Additionally, as can be expected from the Arrhenius equation, the reaction rate increases if the temperature is increased. However, as can be seen in Fig. 3, the equilibrium optical purity also increases at the higher temperature, possibly due to the differing heat stabilities of the diastereomeric salts: the increasing purity of $(S)$-IBU in
the extract suggests that the \((R)\)-IBU–(R)-PhEA salt is more stable at 50 °C. The analogous \((S)\)-IBU–(S)-PhEA salt was reported \cite{10} to have an atmospheric melting point of 187 °C compared to 159 °C for the \((R)\)-IBU–(S)-PhEA salt (analogous to \((S)\)-IBU–(R)-PhEA).

4. Conclusion

The optical resolution of ibuprofen with \((R)\)–(1)-phenylethylamine, based on the modified Pope–Peachey method, was realized using only supercritical carbon dioxide as solvent. Both the formation of the diastereomeric salt and the separation of the unreacted enantiomers from those salts was carried out in the same high-pressure reactor. Diastereomer formation was found to be an equilibrium reaction; hence longer reaction times yield higher efficiencies as the equilibrium point is approached. At 40 °C, increasing the pressure increases the reaction rate (via the negative activation volume) and the equilibrium resolution efficiency (via the more efficient packing of the \((R)\)-ibuprofen–(R)-phenylethylamine salt). At 200 bars, increasing the temperature from 40 °C to 50 °C increases both the reaction rate (via the Arrhenius mechanism) and the equilibrium optical purity (via the differing thermal stabilities of the salts).

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Ammonium carbamate type self-derivative salts of (R-) and racemic \( \alpha \)-methylbenzylamine

Composition and thermal stability by evolved gas analyses (TG–FTIR and TG/DTA–MS)

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Abstract Two different compounds have formed from liquid enantiomeric (R-) and racemic \( \alpha \)-methylbenzylamine (\( \alpha \)-MBA, named also as 1-phenylethylamine, 1-FEA) with supercritical fluid \( \text{CO}_2 \). The crystalline solids have been characterized by elemental CHN analysis, X-ray diffraction (XRD), FTIR, \(^1\)H, and \(^{13}\)C NMR spectroscopy, and found to be \( \alpha \)-methylbenzlammonium \( \alpha \)-methylbenzylcarbamate self-derivative ionic salts 1 \((\mathbf{R}/\mathbf{R})\) and 2 \((\mathbf{r a c R S})\), respectively, of the corresponding amines. Compound 2 \((\mathbf{r a c R S})\) has shown different XRD pattern from that of enantiomerically pure 1 \((\mathbf{R}/\mathbf{R})\), indicating a preferential formation of a 1:1 mixture of \((\mathbf{R}/\mathbf{S})\) and \((\mathbf{S}/\mathbf{R})\) or rather a racemate compound of \((\mathbf{R S} / \mathbf{S R})\) ammonium carbamate salt \((2 \,(\mathbf{r a c R S}))\) from racemate. For thermal stability, the compounds have been checked by differential scanning calorimetry (DSC), simultaneous thermogravimetry and differential thermal analysis (TG/DTA), and in situ coupled evolved gas analysis by mass spectroscopy (TG/DTA–EGA–MS) and FTIR-gas cell (TG–FTIR). No melting point is observed because of the low thermal stability of the compounds. Decomposition stages are tried to be separated with using semi-closed (sealed with a pinhole on the top) crucibles, thus different evolution courses of \( \text{CO}_2 \) and organic vapors could be followed by MS and FTIR spectroscopy. The \( \alpha \)-MBA vapors themselves, evolved from open crucibles could be identified by FTIR-gas cell, while vapors up to \( m/\zeta = 164 \) have been detected by MS from semi-closed Al crucible.

Keywords X-ray diffraction (XRD) · FTIR spectroscopy · Mass spectrometry (MS) · Differential scanning calorimetry (DSC) · Simultaneous TG/DTA · Evolved gas analysis (EGA) · TG/DTA–MS · TG–FTIR

Introduction

Enantiomers (\( \mathbf{R} \)- or \( \mathbf{S} \)-) of \( \alpha \)-methylbenzylamine (\( \alpha \)-MBA, or 1-phenylethylamine, 1-FEA) are frequently applied resolv- ing agents in chiral resolution of various racemic compounds with acidic character through diastereomeric salt formation and fractional crystallization, Kozma [1] lists altogether corresponding 278 cases, about 12.3% of all the racemic acids resolved, according to his comprehensive statistics gathered up to 2002. In general, as a base, \( \alpha \)-MBA is published to form various salts or co-crystals with more than 1,300 acids, including about 120 cases available with structural information on interatomic distances [2]. Anyway, \( \alpha \)-MBAs’ reaction product with \( \text{CO}_2 \), the \( \alpha \)-methylbenzlammonium \( \alpha \)-methylbenzylcarbamate salts (Scheme 1), are not published yet, although they might already have formed at common circumstances, and they may be expected to occur especially in chiral resolutions carried out with supercritical fluid \( \text{CO}_2 \), as solvent [3].

About 90 primary and 25 secondary amines are already well published to react with \( \text{CO}_2 \) and produce self-
ammonium salt of the corresponding carbamic acid formed [2]. Among the aralkyl compounds, which are isomeric, related, or analogous to \( \alpha \)-MBA, the crystal structure of the corresponding self-ammonium carbamate salt of benzylamine [4, 5], 4-methylbenzylamine [6], dibenzylamine [7], and \( (3-(9,10\text{-dihydro-9,10-ethano-9-anthracenyl})\text{propyl})\)benzylamine [8] are already known [9]. The single crystal structure is also reported [9] for self-carbamate salt of several cycloalkylamines, e.g., cyclohexylamine (CSD code: COLQAW), cycloheptylamine (CSD code: COLQEA) [10], 4-amino-4-aza-cyclohexanol [11], piperidine [10, 12–14], 4-methylpiperidine [15], pyrrolidine [16], morpholine [17–20]. Among the chiral amines [9], there are only two, \((1S,2R, 4S,5R)-5\text{-vinyl-1-azabicyclo(2.2.2)oct-2-yl}methylamine [21] and \((1R,2R)-1,2\text{-diaminocyclohexane} [22], for which the crystal structure of this type of chiral ammonium carbamate salt is reported. Among the chiral diamines [9], which form usually zwitterionic ammonium carbamates, the crystal structure of racemic crystals, as rac-endo-endo-N-(5-aminobicyclo(2.2.1)hept-2-yl)carbamic acid hydrate [23] and rac-N-(3-amino-1,5,5-trimethylcyclohexylmethyl) carbamic acid monohydrate [24] are also reported.

Anyhow, no comparative report on both enantiomeric and racemic salts of a particular chiral amine and on their phase relations is available in the literature. Binary phase relation of crystalline racemic:1:1 mixtures to enantiomers [25] is usually established on measurement of melting points and enthalpy changes of fusion [26], beyond structural studies. Nevertheless, such ammonium carbamates are usually decomposed before fusion or melt with decomposition, not allowing such a direct comparison of parameters of melting phenomena.

Here we report preparation of a pair of crystalline self-derivative solid salts of enantiomerically pure and racemic \( \alpha \)-methylbenzylamine formed from the liquid amines with \( \text{CO}_2 \) in supercritical fluid reactor. The composition and thermal stability of the ammonium carbamate type ionic compounds (1 \((R/R)\) and 2 \((\text{racRS})\), respectively, both with 2:1 overall molar ratio of the parent amine and \( \text{CO}_2 \), are studied by elemental CHN analysis, X-ray diffraction (XRD), FTIR, \((^1\text{H} \text{and} ^{13}\text{C})\) NMR spectroscopy, and various thermal (differential scanning calorimetry, DSC, simultaneous thermogravimetry and differential thermal analysis, TG/DSC) and in situ coupled evolved gas analytical methods (EGA–MS, EGA–FTIR), with respect to their formation and decomposition in supercritical fluid \( \text{CO}_2 \) and to their possible phase relation.

### Experimental

Sample preparation and analytical methods

\((R)-(+)\)-phenylethylamine (>99%, Merck, ref. No. 807031, \((R)-\alpha\)-MBA) and racemic \((\pm)\)-MBA (99%, Sigma-Aldrich, ref. No. M31104) were subjected to supercritical fluid \( \text{CO}_2 \) (Linde Gas), at 40 °C, 100 or 200 bar for 3 days. FTIR spectra of the obtained solids, 1 \((R/R)\) and 2 \((\text{racRS})\), were measured by Excalibur Series FTS 3000 (Biorad) FTIR spectrophotometer in KBr in the range of 400–4,000 cm\(^{-1}\). Powder XRD patterns were recorded with a PANalytical X’pert Pro MPD (PANalytical Bv., The Netherlands) multipurpose X-ray diffractometer using Cu \(K\alpha\) radiation, Ni filter, X’celerator detector, and ‘zero background’ single crystal silicon sample holder in the range of 2\(\theta\) = 2–42°.

DSC measurements were performed using a DSC 2920 device (TA Instruments Inc., USA). The samples (1–8 mg) were measured in sealed Al-pans at a heating rate of 10 K/min. According to elemental analysis carried out at the Microanalytical Laboratory of Loránd Eötvös University of Budapest (ELTE-TTK, Budapest, Hungary) on a Vario ELIII (Elementaranalyse) CHN microanalyzer system, the measured elemental composition of 1 \((R/R)\) was found (in mass%): C, 71.15; N, 9.76; and H, 7.71, which is close to that of calculated for \( \text{C}_2\text{H}_{12}\text{N}\text{C}_5\text{H}_{10}\text{NO}_2 \): C, 71.30; N, 9.78; and H, 7.74%.

NMR-spectra were recorded on a DRX-400 (Bruker) spectrometer in DMSO-\(d_6\) at 100.6 and 400.1 MHz for \(^{13}\text{C}\) and \(^1\text{H}\), respectively. In order to confirm the composition and constitution of the compounds further 1D TOCSY, DEPT-135, HSQC (direct \(^1\text{H}–^{13}\text{C}\) correlation), and HMBC (long range \(^1\text{H}–^{13}\text{C}\) correlation) studies were also carried out. For compound 1 \((R/R)\):

![Scheme 1](image-url)
Ammonium carbamate type self-derivative salts

$^1$H NMR (400.1 MHz, DMSO-$d_6$) $\delta$ 7.20–7.37 (m, 10H), 4.63 (m, 1H), 4.19 (s, nN-H), 4.02 (m, 1H), 1.27–1.30 (d, 2 $\times$ 3H). $^{13}$C NMR (100.6 MHz, DMSO-$d_6$) $\delta$ 158.2, 147.3, 146.4, 126.1–128.4, 50.7, 50.1, 25.4, 23.5 ppm.

In situ evolved gas analysis (EGA) by TG–FTIR in open crucible

A TGA 2050 Thermogravimetric Analyzer (TA Instruments, USA) with a heating rate of 10 °C min$^{-1}$, with air flow rate of 120 mL/min (and an extra 10 mL/min air as a balance purge), and sample size 6–72 mg of samples in open Pt crucible were used. Gaseous species evolved from the sample were led into FTIR-gas cell of the BioRad TGA/IR Accessory Unit equipped with Peltier-cooled DTGS detector through a heated stainless steel transfer line ($l = 90$ cm, $d_{in} = 2$ mm) kept at $T = 180$ °C. FTIR spectra (500–4,000 cm$^{-1}$) were collected in every 30 s after accumulation of 29 interferograms by a BioRad Excalibur Series FTS 3000 spectrometer using Win IR Pro 2.7 FTIR (BioRad) data collection and evaluation software.

In situ EGA by coupled TG/DTA–MS in semi-closed crucible

A simultaneous TG/DTA apparatus (STD 2960 Simultaneous DTA-TGA, TA Instruments Inc., USA), a heating rate of 10 °C min$^{-1}$, an air flow rate of 130 mL/min, sample sizes between 1 and 11 mg, and sealed Al crucible with a pinhole on the top was used. The mixture of gaseous species could reach the ThermosStar GDS 200 (Balzers Instruments) quadrupole mass spectrometer equipped with Channeltron detector, through a heated 100% methyl deactivated fused silica capillary tubing kept at $T = 200$ °C. Data collection was carried out with QuadStar 422v7.02 software in scanning (SCAN) mode in the range of $m/z = 1–300$ (0.2 s/channel) and also in Multiple Ion Detection (MID) mode monitoring only 64 selected channels based on changes observed in scanning mode. Measuring time was ca. 0.5 s for one channel, resulting in time of measuring of each MID cycle in ca. 32 s.

Results and discussion

Compositional characterizations by XRD, FTIR, and NMR spectroscopies

The XRD patterns of the solid samples 1 (RR) and 2 (racRS) prepared under 200 bar CO$_2$ from the liquid amines are shown in Fig. 1 in comparison. The numerical peak data deposited as Tables 1 and 2 in the Supplementary materials also show that two different crystalline entities formed from the enantiomeric and racemic amines, respectively. Each pattern is unchanged even if the samples are prepared at 100 bar or at ambient pressure, so they are not pressure modifications of each other. Most likely, the different XRD patterns indicate a preferential formation of a 1:1 mixture of (R/S-) and (S/R-) or rather a racemate compound of (RS/SR-) ammonium carbamate salt (2 (racRS)) starting from racemate amine, rather than the homochiral compound 1 (RR) and its mirror image 1 (SS).

Fig. 1 XRD patterns of samples 1 (RR) (bottom, a) and 2 (racRS) (top, b) prepared under 200 bar, in comparison. The significant difference of the patterns indicates different crystalline entities starting from racemate amine, rather than the homochiral compound 1 (RR) and its mirror image 1 (SS).

The FTIR spectra of the samples 1 (RR) and 2 (racRS) prepared under 100 bar are presented in Fig. 2, in comparison. The spectra are similar but not the same. Both indicates the formation ammonium carbamate type salts, exhibiting an absorption band of $\nu$(N–H) stretching vibration of secondary amine (>NH) group in a carbamate anion peaked at 3,371 and 3,383 cm$^{-1}$ for 1 (RR) and 2 (racRS), respectively (see similar features in spectra of benzylcarbamic acid benzylamine salt [27]). A significant shoulder, a duplication of $\nu$(N–H) band, at around 3,334 cm$^{-1}$ in the spectrum of 1 (RR) indicates, that the (R)-x-methylbenzylcarbamate are located anions are located in two crystallographically different positions or neighborhoods. There is also a broad hydrogen bonding system, similar in each sample, between 3,200 and 2,000 cm$^{-1}$, where an indicative, largely down-shifted band of primary ammonium group (R-NH$_3^+$) vibrations occurs also between 2,100 and 2,250 cm$^{-1}$ in both cases, however, there are
slight differences in the patterns of these band systems of secondary hydrogen bondings, as well.

Structural confirmation of ammonium carbamate salt formation was performed by NMR spectroscopies. There are two separate signals of aliphatic methin (ternary \( \equiv \text{CH} \)) group present in both the \(^1\text{H}\) and \(^{13}\text{C}\) spectra, peaks at \( \delta\text{H} = 4.62 \) and \( \delta\text{C} = 50.1 \) ppm can be assigned to the benzylic CH group of the ammonium cation, while peaks at \( \delta\text{H} = 4.02 \) and \( \delta\text{C} = 50.7 \) ppm to that of carbamate anion. The evaluation of HSQC (direct \(^1\text{H} - ^{13}\text{C}\) correlation, Fig. 3) and HMBC (long range \(^1\text{H} - ^{13}\text{C}\) correlation, not shown) spectra confirms the presence of two entities and completes their structural assignment.

Thermal stability characterization by DSC

Actually, no sharp melting points of crystalline salts 1 \((R/R)\) and 2 \((\text{racRS})\) are observed in the hermetically closed Al crucibles, instead one relatively broad decomposition endothermic heat effect has occurred, which is peaked at 104 and 108 °C, respectively, independently on the applied preparation pressure (100 or 200 bar). It may indicate a slightly higher thermal stability of racemic 2 \((\text{racRS})\) salt. Anyhow, the difference in the peak temperatures might be also assigned to the slight differences in the density of samples. (Note: A mixture of the two salts formed from a 1:1 mass ratio of enantiomeric to racemic amines, i.e., 50\%\enantiomer excess for the favor of \((R)-\varepsilon\text{-MBA}, are originally found very stuff and dense with a DSC heat effect at 119 °C, which sample after storage at 30 °C for a night showed only a peak centered at 101 °C.)

Thermal decomposition in open Pt crucible traced by TG–EGA–FTIR

The thermal decomposition of these ammonium carbamate salts, 1 \((R/R)\) or 2 \((\text{racRS})\), is basically a reversible single-step heterogeneous reaction:

\[
1(R/R)_s \rightleftharpoons (R)-\varepsilon\text{-MBA}(_g) + \text{CO}_2(_g),
\]

whose overall kinetics is sensitive to the various effects of heat and mass transport processes, demonstrated by the quasi single-step decomposition observed in open Pt pans started with various initial sample amounts (Fig. 4), when in each case, however, unsmooth DTG curves are observed with some discrepancies. When the initial mass of 1 \((R/R)\) and 2 \((\text{racRS})\) is almost the same, the curves are strikingly overlaying ones, indicating no significant difference in the thermal stability of the two solids 1 \((R/R)\) and 2 \((\text{racRS})\).

The roughness of the DTG curves can be explained both by reversibility of reaction and the significant difference in the volatility of the two decomposition products, \text{CO}_2 gas and liquid \(\varepsilon\text{-MBA} (bp. 180–187 °C) [28, 29]. It could also be observed in form of differently peaked evolution curves when the two products have been traced in situ by EGA with FTIR-gas cell (Fig. 5). The reference IR spectra of \(\varepsilon\text{-MBA vapor have been obtained from the NIST/EPA Gas-Phase Infrared Database [30], with which the evolved vapor could be identified without doubts (Fig. 6).}
The thermal decomposition of the ammonium carbamate salts, 1 (R/R) or 2 (racRS), in a sealed but with a pinhole on the top Al crucible has been effected much more with the self-generated atmosphere of CO$_2$ and α-MBA. Depending on the size of the pinhole, various separations of decomposition and evaporation processes could be reached (Fig. 7) into two steps, to some extent. Thus, even a comparison with the theoretical mass loss corresponding to the evolution of stoichiometric amount of CO$_2$ (15.37%) could be done, as well as separate endothermic heat effects have been observed for the two release steps.

Application of the self-generated atmosphere and the semi-closed Al pan resulted also in some degradation side reaction of α-MBA (or a catalytic side-effect), as the products were followed in situ by TG/DTA–EGA–MS. In these cases, variable amount (1–7%) of final mass residues could be obtained (Fig. 7, TG curves). In the mass spectra of the evolved gases, various ion fragments with mass numbers ($m/z = 134, 148, 164$) higher than $m/z = 121$ (i.e., molecular mass of α-MBA [31]) have also occurred indicating additional formation of volatile condensation product(s) beyond the α-MBA (Fig. 8). Actually, the mass spectroscopic gas-evolution curves, i.e., the ion current

![Figure 4: Thermal decomposition (TG and DTG) curves of 1 (R/R) in open Pt pans with five gradually increasing initial sample mass, and in addition those of 2 (racRS) in one case.](image)

![Figure 5: Evolution curves of CO$_2$ gas (top) and α-MBA vapor (bottom), as integrated absorbance versus temperature curves of identified gaseous species evolved from ammonium carbamate salt, 1 (R/R) measured in open Pt crucible by online coupled TG–FTIR system (heating rate 10 °C min$^{-1}$, initial mass 6.965 mg).](image)

![Figure 6: IR spectra of experimentally evolved α-MBA vapor from 1 (R/R) (top) at 163 °C and its NIST/EPA reference gas-phase spectrum (bottom) [30].](image)
curves of various characteristic ion fragments of both α-MBA and the unidentified vapors released from the samples, have run parallel versus time/temperature as detected by the online coupled TG/DTA–EGA–MS system (Fig. 9).

A temporary formation of aziridine (C$_2$H$_2$N) as partial degradation product of α-MBA and its amine-chain- extending reaction with unchanged α-MBA are supposed [32]. However, neither the intermediate aziridine nor its suggested product could be traced by TG–FTIR.

Conclusions

Novel crystalline self-derivative solid salts of enantiomerically pure and racemic α-MBA (or 1-phenylethylamine, FEA) have been formed from the liquid amines with CO$_2$ in supercritical reactor. They are potential by-products in chiral resolutions with the amine as resolving agent to be carried out using supercritical fluid CO$_2$ as solvent. The ammonium carbamate type ionic compounds (1-R/R and 2-racRS, respectively), both with 2:1 molar ratio of the parent amine and CO$_2$, have been characterized with their own XRD pattern (XRD), FTIR-spectrum, and NMR-spectra ($^1$H and $^{13}$C). The parent carbamic acid is the simplest acidic derivative of α-MBA (1-FEA). The chiral amine was successfully resolved (among others) by its own monoacidic monoamide self-derivatives formed with dicarboxylic acids, as oxalic, malonic, succinic, and glutaric acids, in the form of self-oxalamic/malonamic/succinamic acids, in the form of self-oxalamic/malonamic/succinamic and glutaramic acid α-MBA (1-FEA) diastereoisomeric salts [33–35]. Compound 2-racRS has shown different XRD pattern then that of enantiomerically pure 1-R/R, indicating a preferential formation of RS- and SR- (or complex RS/SR-) ammonium carbamate salt (2-racRS) from racemic α-MBA (1-FEA). Actually the thermal stability of the two salts are very close, determined by the strength of primary chemical bonds, and the overall strength secondary hydrogen bonding system seems to be also quite similar to each other. Because of this preferential formation of 2-racRS, no spontaneous resolution of α-MBA (1-FEA) can be expected during the formation of these ammonium salts.
Ammonium carbamate type self-derivative salts

Carbamate salts in the presence of CO₂, what was not mentioned in the literature yet. Application of enantiomERICALLY pure carbamic acid of z-MBA (1-FEA) in resolution of other racemic amines is depending on whether a formation of mixed type ammonium carbamate is thermodynamically or kinetically preferred or not [34]. Anyhow, we have found the combined usage of TG/DTA-MS and TG-FTIR methods useful, complementary, and confirming, like in former studies [36–38].

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References

Crystallization and Resolution of cis-Permethric Acid with Carbon Dioxide Antisolvent

Crystallization and resolution of cis-permethric acid (cPA) with (1R)-1-phenylethanamine (PhEA) as a resolving agent was investigated by means of gas antisolvent (GAS) and supercritical antisolvent (SAS) methods. A significant pressure effect on both the yields and diastereomeric excesses of the crystallized cPA-PhEA salts was observed in a defined pressure range. The pressure effect was found to be related to the structure and dissociation of the salts. Both methods yielded diastereomeric salts with excellent diastereoselectivity, compared to data reported in literature, and a fibrous structure of uniform fiber diameter.

Keywords: Diastereomeric salt crystallization, Gas antisolvent process, Supercritical antisolvent process, Supercritical carbon dioxide

1 Introduction

The production of enantiopure compounds is at the forefront of interest in contemporary chemical engineering [1], especially in the pharmaceutical, pesticide, and food industries. The rising demand for chiral molecules requires that their production be both economically viable and environmentally safe.

Supercritical carbon dioxide (scCO2) is an established industrial solvent with many environmentally desirable properties, i.e., nonflammable, nonexplosive, nontoxic in trace amounts, readily available, etc. Supercritical fluid extraction (SFE) with CO2 has been successfully used in resolutions of various compounds [2–4]. The racemic compound and a chiral resolving agent are dissolved in a conventional solvent. The resolving agent is added in half-molar equivalent quantity, according to the Pope-Peachey method [5]. The organic solvent is evaporated, yielding diastereomeric salts enriched in one enantiomer, and unreacted enantiomers enriched in the antipode. The mixture of unreacted enantiomers is separated from the polar, highly scCO2-insoluble salts by SFE. Chiral resolution has been also performed with scCO2 as an antisolvent [6,7]. Antisolvent precipitation with scCO2 gained significant scientific interest in recent years, and production-scale implementations are envisioned [8,9].

In this paper, resolution and crystallization of racemic cis-permethric acid (IUPAC name: 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylic acid, abbreviated cPA) using (1R)-1-phenylethanamine (PhEA) as a resolving agent is presented. The idealized reaction scheme is illustrated in Fig. 1. According to the modified Pope-Peachey method, PhEA is added to cPA in half mole equivalent ratio and the diastereomeric salt precipitates. Previously reported results [10] on this resolution system, using scCO2 extraction, found that the (–)-enantiomer of cPA forms a diastereomeric salt with PhEA which is not dissolved in scCO2, while the unreacted (+)-enantiomer appears in the extract. This is, of course, an idealized view of the reaction, as both the diastereomeric salts and the unreacted enantiomers contain some amount of the antipode.

Resolution and crystallization are performed via two antisolvent crystallization techniques: the batch gas antisolvent (GAS) and the semi-continuous supercritical antisolvent (SAS) methods. Note that despite the designation both methods use scCO2.

Figure 1. Idealized reaction scheme of the resolution of cPA with PhEA according to the modified Pope-Peachey method.
2 Materials and Methods

2.1 Materials

CO₂ (99.5 %) was purchased from Linde Gas Hungary Co. (Budapest, Hungary). (1R)-1-Phenylethanolamine (99 %, GC) was from Merck Ltd. (Budapest, Hungary). Racemic and enantiopure cis-permethric acid was kindly given by Chinoin Ltd. (Budapest, Hungary). Methanol (99.9 %, GC) was purchased from Sigma-Aldrich Hungary Ltd. (Budapest, Hungary).

2.2 Experimental Methods

The GAS experiments were carried out in a high-pressure reactor as previously described [11] in our work concerning in situ diastereomer formation. Unless otherwise stated, for the experiments 130 ± 0.5 mg (0.62 ± 0.002 mmol) racemic cPA and 37.6 ± 0.5 mg (0.31 ± 0.004 mmol) PhEA were dissolved in 2 ± 0.008 mL methanol, and the solution was filled into the tempered reactor vessel. The reactor was sealed and filled with CO₂ until the desired pressure was reached, at which point the stirring was activated for 1 h. Then, the reactor was washed with 90 mL scCO₂ at the pressure and temperature of the reactor in order to extract the CO₂-soluble components and collect them in the liquid trap, here methanol. After depressurization during which CO₂ leaving the reactor was passed through the liquid trap, the reactor was washed with 90 mL methanol. The ratio between the resolving agent and the racemic compound is described by the molar ratio (mr) according to the following relationship:

\[ mr = \frac{n_{\text{res}}}{n_{\text{rac}}} \]  

where \( n \) denotes the molar quantity and the indices res and rac refer to the resolving agent and the racemic compound, respectively. Earlier work by Keszei [12] showed that \( mr = 0.5 \) is optimal for the resolution of cPA, thus, unless stated otherwise, all samples have been prepared at this molar ratio.

The optical purity of products was characterized by the enantiomeric excess (ee). In the experiments, enantiomeric excesses were calculated from peak areas obtained by gas chromatography:

\[ \text{ee} = \frac{A_{(+)-cPA} - A_{(-)-cPA}}{A_{(+)-cPA} + A_{(-)-cPA}} \]  

where \( A \) refers to areas below the peak and the configuration of the enantiomer is indicated in the indices. Values of ee range from 0 for a racemic compound to 1 for a pure enantiomer. The diastereomeric salts decompose at the temperature of the chromatographic analysis, thus the ee of cPA is measured for both the extract and the raffinate. However, due to the exhaustive washing with CO₂, it can be assumed that only the polar salts containing a 1:1 ratio of cPA and PhEA remain in the raffinate, thus the ee of cPA measured in the raffinate is equal to the diastereomeric excess (de) of the cPA-PhEA salt.

If the diastereomer formation proceeds to 100 % conversion and the extraction removes the unreacted enantiomers completely, the raffinate will contain half the mass of cPA and the entire mass of PhEA. Therefore, raffinate yields (\( Y_r \)) were calculated by the formula:

\[ Y_r = \frac{m_r}{m_{\text{cPA}}} + \frac{m_{\text{PhEA}}}{2} \]  

where \( m \) denotes the mass, the index r refers to the raffinate, while indices cPA and PhEA refer to the respective materials. The overall resolution efficiency can be described by the selectivity (S) as defined by Fogassy et al. [13]. For the raffinate, it can be calculated by the formula:

\[ S_r = Y_r \times e e_r \]  

The selectivity parameter ranges from 0 to 1, the value of 1 representing a perfect resolution.

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1) List of symbols at the end of the paper.
2.3 Analytical Methods

The enantiomeric excesses of samples were determined by GC on a Thermo Finnigan Focus GC (Thermo Fisher Scientific, Waltham, MA, USA) fitted with a Supelco BetaDEX 120 column and an FID detector at 250 °C. Temperature program: 1 min at 110 °C, 10 °C min⁻¹, 3 min at 200 °C, 10 °C min⁻¹, 4 min at 230 °C. Carrier gas (He) pressure was 170 kPa. Injections were done manually with an injection volume of 0.6 μL.

Powder X-ray diffraction (XRD) measurements were done on a PANalytical X′Pert Pro MPD diffractometer (PANalytical, Almelo, The Netherlands), equipped with an Xcelerator detector in θ-θ arrangement to the beam source, at the Cu Kα wavelength (1.5408 Å) applying 40 kV tension and 30 mA current. Diffractograms were recorded in the 4°–42° range, with a 1 minute-of-angle (0.0167°) step size and 31.115 s counting time. Scanning electron microscope (SEM) images were recorded by a JEOL JSM 5500-LV scanning electron microscope using 20 kV voltage and a secondary electron detector. For SEM studies, the samples were covered with a 5–10 nm Au/Pd layer to make them conductive.

3 Results and Discussion

3.1 Gas Antisolvent (GAS) Crystallization

The effect of pressure on the diastereomeric salts was studied in detail at 45 °C between 100 and 200 bar. Experiments were conducted at 10-bar increments. Fig. 3 presents the results of these experiments. According to Fig. 3a, the raffinate yields show a decreasing trend between 100 and 170 bar. At higher pressures, virtually no raffinate is recovered, the yield results from non-ideal washing. Raffinate de exhibits three distinct regions: very low de (≤ 5 %) between 100 and 120 bar, excellent de (> 80 %) between 130 and 170 bar, and moderate de (20–40 %) between 180 and 200 bar. The selectivity parameter S, indicated in Fig. 3b, shows the net effect of the de and yield: where either is near zero (100–120 bar, 180–200 bar), the selectivity is also extremely low (< 0.05). Between 130 and 170 bar, the selectivity shows a slightly decreasing linear trend, from 0.36 to 0.25.

Since such a sharp pressure effect during GAS crystallization has not been reported previously in the literature, the diastereomeric salts were further investigated using XRD measurements and independent crystallization experiments. For the XRD measurements, equimolar cPA-PhEA standards were prepared from racemic (+)- and (-)-cPA by dissolving both components in methanol, followed by evaporation of the solvent in vacuum. Diffractograms of raffinates obtained by the GAS process between 130 and 170 bar were in good agreement with the (-)-cPA-PhEA standard. Diffrac togroms of raffinates produced between 100 and 120 bar were consistent with each other, however, as demonstrated in Fig. 4, they were not the superposition of the diffractograms of (-)-cPA-PhEA and (+)-cPA-PhEA, which would indicate a physical mixture of the two diastereomers. Additionally, diffractograms indicated that the raffinates contained no pure enantiomers or racemic cPA. However, the diffractograms of these raffinates agreed with the diffractogram of the standard prepared from racemic cPA. This

![Figure 3](image-url)  Figure 3. Effect of pressure on (a) raffinate yield and diastereomeric excess towards (-)-cPA-PhEA; (b) resolution efficiency in the GAS resolution of cPA with PhEA at 45 °C.

![Figure 4](image-url)  Figure 4. X-Ray diffractograms of a raffinate obtained by the GAS process at 110 bar, 45 °C (1), compared to pure diastereomer standards (2, 3).
suggests that the racemic salt is stable between 100 and 120 bar, the (-)-cPA salt is stable between 130 and 170 bar, and no salt is stable between 180 and 200 bar.

Independent crystallization experiments were conducted to explain the excellent selectivity observed in the 130–170 bar region, the results of which are summarized in Tab. 1. Column A shows the details of an experiment performed to study the effects of pressure, presented to provide reference values. Column B displays the results of experiments in which racemic cPA was replaced with the pure enantiomers, keeping the half-molar ratio. Both enantiomers formed stable salts between 100 and 120 bar; however, XRD results detailed earlier proved that the racemic salt is more stable than either pure diastereomer. Between 130 and 170 bar, only (-)-cPA yielded a raffinate, thus affirming that the excellent selectivity in this region is due to the differing stability of the diastereomeric salts. At 180–200 bar, no salts were obtained with either enantiomer. Column C indicates the results of equimolar experiments. Comparing columns B and C shows that in a defined pressure range, for a given enantiomer, the half-molar experiments resulted in a higher yield of salt than the equimolar experiments. As the amount of recoverable salt is higher when cPA is in excess, it can be concluded that the salts dissolve by dissociation.

<table>
<thead>
<tr>
<th>mr</th>
<th>n_{cPA} [mmol]</th>
<th>n_{PhEA} [mmol]</th>
<th>cPA configuration</th>
<th>Y_r at 100–120 bar</th>
<th>Y_r at 130–170 bar</th>
<th>Y_r at 180–200 bar</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>0.62</td>
<td>0.31</td>
<td>Racemic (+)</td>
<td>0.40–0.65</td>
<td>0.30–0.60</td>
<td>&lt; 0.10</td>
</tr>
<tr>
<td>0.5</td>
<td>0.62</td>
<td>0.31</td>
<td>(-) (–)</td>
<td>0.40</td>
<td>0.09</td>
<td>&lt; 0.10</td>
</tr>
<tr>
<td>1</td>
<td>0.31</td>
<td>0.31</td>
<td>(+) (–)</td>
<td>0.47</td>
<td>0.15</td>
<td>&lt; 0.10</td>
</tr>
</tbody>
</table>

From the XRD measurements and independent crystallization experiments, it is inferred that the effect of pressure on the resolution is due to the structure of the diastereomeric salts as well as their dissociation, summarized in Fig. 5. Between 100 and 120 bar, the racemic salt is the most stable as confirmed by XRD, thus raffinates in this region have extremely low de. Between 130 and 170 bar, the (+)-cPA-PhEA salt dissociates more readily than (-)-cPA-PhEA, causing high de. Between 180 and 200 bar, both salts dissociate, causing the raffinate yield to decrease to near zero.

3.2 Supercritical Antisolvent (SAS) Crystallization

GAS crystallization suffers from the low productivity inherent to batch processes. Therefore, it was attempted to realize the crystallization in a semi-continuous SAS process, under the conditions found to be optimal for GAS experiments. In these tests, 1500 ± 0.5 mg (7.17 ± 0.002 mmol) cPA and 434.7 ± 0.5 mg (3.59 ± 0.004 mmol) PhEA were dissolved in 7.5 ± 0.02 mL methanol. The crystallizer vessel was tempered to 45 °C and pressurized to 150 bar, which corresponds to conditions found to be optimal during the GAS experiments, and the solution was injected into the vessel. The crystallized salts, i.e., the raffinate, were recovered from the crystallizer in solid form, while the unreacted enantiomers and the methanol, i.e., the extract, were recovered as a solution from the separator.

The de values of the raffinates obtained in the SAS experiments was generally comparable to those obtained by the GAS process between 130 and 170 bar (> 85 %) and in some experiments reached 95 % de ((–)-cPA-PhEA). Yields were slightly lower than those in the GAS experiments (10–25 %). SAS raffinates with lower yields typically had higher de values and vice versa.

The low bulk density of ~ 5 kg m⁻³ compared to ~ 100 kg m⁻³ for XRD standards of both GAS and SAS raffinates prompted us to study them by SEM, the results of which are shown in Fig. 6. Both methods yielded diastereomeric salts consisting of fibers with diameters varying between 500 and 700 nm and lengths exceeding 100 μm. Fiber diameters varied with the method and the pressure, but were consistent within samples.

4 Conclusions

A method for the crystallization and resolution of racemic cPA with PhEA as a resolving agent was developed. The crystallized products provided yields similar to those previously reported...
in the literature, i.e., 10–40% compared to 34% [10], but the diastereomeric excess in a single step exceeded those reported earlier being 85–95% (−)-cPA-PhEA compared to 74% [10]. A significant pressure effect was observed which could not be explained by solubility effects alone. Salt structure and dissociation are assumed as the key factors. Between 100 and 120 bar, racemic cPA forms the most stable salt with PhEA, so almost no de is observed. Between 130 and 170 bar, the (+)-cPA-PhEA salt dissociates while the antipode is stable, leading to excellent diastereoselectivity. Between 180 and 200 bar, both salts dissociate, leading to virtually no salt yield.

The batch GAS process was successfully realized as a semi-continuous SAS process, which involved a ten-fold scale-up with respect to the mass of cPA. The crystallized salt had an excellent diastereomeric excess of up to 95% (−)-cPA-PhEA and a uniform fibrous structure with very high length-to-diameter ratios of 500–700 nm diameter versus several tens of micrometers length.

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The authors have declared no conflict of interest.

Symbols used

\[
\begin{align*}
A & \quad [\text{mV s}] \quad \text{area under curve on chromatogram} \\
de & \quad [-] \quad \text{diastereomeric excess} \\
\text{ee} & \quad [-] \quad \text{enantiomeric excess} \\
m & \quad [\text{g}] \quad \text{mass} \\
mr & \quad [-] \quad \text{molar ratio} \\
n & \quad [\text{mol}] \quad \text{molar quantity} \\
S & \quad [-] \quad \text{selectivity} \\
Y & \quad [-] \quad \text{yield}
\end{align*}
\]

Subscripts

\[
\begin{align*}
r & \quad \text{raffinate} \\
rac & \quad \text{racemic material} \\
res & \quad \text{resolving agent}
\end{align*}
\]

Abbreviations

\[
\begin{align*}
\text{GAS} & \quad \text{gas antisolvent} \\
\text{SAS} & \quad \text{supercritical antisolvent} \\
\text{SEM} & \quad \text{scanning electron microscopy} \\
\text{XRD} & \quad \text{powder X-ray diffraction}
\end{align*}
\]

References

Chiral Resolution of Racemic Cyclopropanecarboxylic Acids in Supercritical Carbon Dioxide

The chiral resolution of two racemic cyclopropanecarboxylic acids with the resolving agents \((S)-2-(N\text{-benzylamino})\text{butan-1-ol}\) and \((R)-1\text{-phenylethanamine}\) was investigated. The resolutions were based on diastereomer salt crystallization in supercritical carbon dioxide. Unreacted compounds were removed by an extraction step. Experiments were performed in a continuously stirred tank reactor and good enantioselectivities were obtained in a single step. Pressure, temperature, and density were found to strongly affect both the optical purity and the selectivity of the resolutions. The crystal structures of the formed diastereomer salts were also studied via X-ray diffraction.

Keywords: Chiral resolution, Diastereomer salt formation, Enantiomer separation, Supercritical carbon dioxide

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1 Introduction

The preparation of pure enantiomers is a key issue in the chemical industry. Typical large-scale resolutions are mainly based on diastereomeric salt formation followed by fractionated crystallization [1]. One of the possible diastereomeric salt formation-based resolution techniques is the modified Pope-Peachy method, where the resolving agent is added in half-mole equivalent quantity to the racemic compound and no nonchiral acid or base is added to keep the nonreacted enantiomer in the solution. When the enantiomers are soluble in a supercritical fluid, fractionated crystallization can be replaced by supercritical fluid extraction (SFE) in order to separate the enantiomers [2]. Furthermore, as is the case in our work, supercritical solvents can be used as a reaction medium as well. 3-(2,2-Dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (cis-permethric acid, CPA) is a precursor, metabolite, and environmental degradation product of the pyrethroid-type insecticide permethrin [3]. The resolution could be achieved via a two-step chromatographic process combining a diastereoselective reverse-phase separation in the first step with a direct enantiomer separation in the second step. The enzymatic resolution of CPA is also already published in the literature [4].

Deltamethrin is known as one of the most effective insecticides which can be obtained from 3-(2,2-dimethylvinyl)-2,2-dimethylcyclopropanecarboxylic acid (cis-chrysanthemic acid, CCA). It is approximately 100 times more effective than 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane, known as DDT, but not poisonous to homeothermic animals and easily biodegradable [5].
Simándi et al. [2] found that the resolving agents (S)-2-(N-benzylamino)butan-1-ol (BAB) and (R)-1-phenylethanolamine (PhEA) were applicable to the resolution of racemic perimethic acids (both cis- and trans-permethic acids) using the modified Pope-Peachy method by crystallizing the diastereomeric salt from an organic solvent with vacuum evaporation (in vacuo method) followed by SFE. They observed that the extraction conditions in terms of pressure and temperature strongly affect the enantiomeric excess (ee) of the products. The assumption was that the change of ee as a function of extraction conditions can only be caused by a chemical reaction taking place during extraction. Bánsgáhi et al. successfully carried out the in situ diastereomer salt formation reaction of ibuprofen with PhEA in supercritical carbon dioxide (scCO₂).

The assumption was that the change of ee as a function of extraction conditions can only be caused by a chemical reaction taking place during extraction. Bánsgáhi et al. successfully carried out the in situ diastereomer salt formation reaction of ibuprofen with PhEA in supercritical carbon dioxide (scCO₂). The diastereomeric salt formation reaction was slow; the equilibrium was reached in approximately one week [6]. A much faster reaction system is presented as well as comparisons of the in vacuo and in situ methods to reach the enantiomer separation both of CPA and CCA in scCO₂.

2 Materials and Methods

2.1 Novel Method for BAB Synthesis

The 10 % Pd/C (Selcat Q) catalyst was manufactured according to a patented method [7] by Szilor Fine Chemicals (Budapest, Hungary). The dispersion of the catalyst, determined by H₂, O₂, and CO chemisorption measurements, is D = 50 %, Benzaldehyde (99 %) and (S)-2-aminobutan-1-ol (98 %) were supplied by Sigma-Aldrich (Steinheim), while toluene (p.a.) and hexane (p.a.) were purchased from Merck-Schuchardt (Darmstadt). The reaction equation is presented in Fig. 1.

A solution of benzaldehyde (5.30 g, 0.05 mol) in 20 cm³ toluene was added to a solution of (+)-(S)-2-aminobutan-1-ol (4.45 g, 0.05 mol) in 40 cm³ toluene. The mixture was stirred at 50 °C for 10 min. After cooling, it was evaporated under vacuum to obtain 8.78 g (0.0496 mol) crude product with 99.2 % yield, as a yellowish oil. This intermediate Schiff base was hydrogenated over a 10 % Pd/C catalyst (0.9 g, Selcat Q) in 100 cm³ toluene at room temperature using a conventional atmospheric pressure apparatus equipped with a 250-cm³ flask and a magnetic stirrer with a stirring speed of 1100 rpm. After finishing the hydrogen uptake, typically requiring 1.5 h reaction time, the catalyst was filtered off and the filtrate was evaporated under vacuum. The amount of the pale yellow solid crude product was 7.41 g (82.8 %). After recrystallization from hexane, BAB was obtained as a white solid with the following characteristics: m.p. 71–72°C (72°C in [8]); GC-MS m/z (rel %) 178(1), 148(30), 91(100), 70(12), 65(18); +23.3 (c 0.9, EtOH).

2.2 Other Materials and Reagents

The racemic acids (purities > 99 %, GC-MS) were kindly given by Chinoin Ltd (Budapest, Hungary). PhEA (> 99 %, GC) and solvents were purchased from Merck Ltd. (Budapest, Hungary). Carbon dioxide (99.5 %) was obtained from Linde Gas Hungary Co. (Budapest, Hungary). The inert porous supporting material (Perfil 250) was kindly provided by Baumit Ltd. (Budapest, Hungary).

2.3 In Vacuo Diastereomer Salt Crystallization

In the in vacuo method, the diastereomer salt was crystallized from an organic solvent under vacuum in two steps. First, the acid and the resolving agent were dissolved in an organic solvent, i.e., methanol and dichloromethane for CPA and CCA, respectively, the solutions were united, and the solvent was evaporated in 50–200 mbar vacuum at 40–45 °C temperature. Solid, crystalline samples could be gathered only when a porous supporting material was employed. Since earlier experiments [2] proved it to be inert, Perfil 250 was used. The formed salt was placed in the tempered reactor, which was then sealed and filled with scCO₂ to the desired pressure. Reaction mixtures were sampled at various times between 1 h and 40 h. The sampling method and further experimental details are identical to those presented at the end of Sect. 2.4.

2.4 In Situ Experiments

In situ salt formation reactions, the organic solvent was omitted entirely in favor of scCO₂: starting materials, i.e., the racemic acid and the resolving agent, were measured into the reactor without any preparation. No Perfil 250 was used in these experiments. The high-pressure reactor and its operation were described by Bánsgáhi et al. [6]. Fig. 2 displays a graphic representation of the two methods and the entire resolution process.

Figure 1. Synthesis of (S)-2-(N-benzylamino)butan-1-ol (BAB).
In experiments performed with CPA, 400 ± 0.5 mg of CPA and 155 ± 0.5 mg of BAB (considering its purity of 97.11 %) or 100 ± 0.5 mg PhEA were measured into the reactor. With these amounts, the initial molar ratios (Eq. (1)) were $m^{+} = 0.45$ for BAB and $m^{-} = 0.43$ for PhEA. In experiments performed with CCA, 150 ± 0.5 mg of acid and 72 ± 0.5 mg of BAB were measured into the reactor, thus the initial molar ratio was $m^{-} = 0.45$. The reactor was filled with CO$_2$ to the desired pressure. The useful reactor volume, i.e., the efficiently stirred volume where reaction takes place, was determined to be 33 mL. The stirring rate was set so that the reactor was turbulently stirred with Re > 10 000 taking into account the geometry of the reactor and the applied stirrer. Experiments were run for about 4 h. At selected reaction times, mostly at 1, 2, and 3 h, three samples were taken from the CO$_2$ phase, each of them with 2.3 mL scCO$_2$. One sample contained 15–35 mg of extract, depending on the applied acid and resolving agent. After taking the last sample, the reactor was washed out with 60 mL scCO$_2$. The stream of scCO$_2$ leaving the reactor was expanded to atmospheric pressure and passed through a liquid trap which captured the CO$_2$-soluble components, i.e., the extract. CO$_2$-insoluble components, i.e., the raffinate, remained in the reactor vessel.

### 2.5 Analytical Methods

GC-MS analyses were carried out with a Finnigan Mat/Autospec II GC/MS spectrometer using a Zebrom ZB-5ms capillary column with 30 m × 0.25 mm ID and 0.25 μm film. The temperature program was as follows: 45 °C for 2 min to 300 °C at 10 °C min$^{-1}$, then to 350 °C in steps of 25 °C min$^{-1}$.

Melting points were taken using a MEL-TEMP apparatus and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 automatic polarimeter.

Enantiomeric excess values were determined by GC on a Thermo Finnigan Focus GC (Thermo Fischer Scientific, Wall-tham, MA, USA) equipped with a Supelco BetaDex 120 FS chiral column with 30 m length and 0.250 mm ID and detected by an FID at 250 °C. One microliter of the sample was injected manually by a Hamilton syringe. The carrier gas was helium (99.99 % purity).

XRD measurements were done on a PANalytical X’Pert Pro MPD (PANalytical, Almelo, The Netherlands) diffractometer, equipped with an X’celerator detector in θ-θ arrangement to the beam source. Measurements were performed at the Cu K$_\alpha$ wavelength (1.5408 Å) applying 40 kV tension and 30 mA current.

### 2.6 Extraction Parameters

The relative amount of the racemic acid and the resolving agent is described by the molar ratio:

$$m'r = \frac{n_{res}}{n_{rac}}$$  

where $n$ denotes the molar quantity and indices res and rac refer to the resolving agent and the racemic compound, respectively.

Since, theoretically, the extract could only contain one of the enantiomers, the yield ($Y_E$) is calculated from the mass recovered in the extract ($m_E$) divided by the unreacted amount of the racemic acid:

$$Y_E = \frac{m_E}{m_{rac}(1 - m'r)}$$  

where $m_{rac}$ denotes the initial mass of the racemic acid. The yield of the raffinate was calculated by defining a theoretical raffinate mass at full conversion:

$$m_{R} = m'r m_{rac} + m_{res}$$

Thus, $m_R$ is the theoretical maximum weight of the diastereomer salt. The yield of the raffinate ($Y_R$) was then calculated by dividing the actual recovered raffinate mass ($m_R$) minus the mass of Perfil ($m_p$) by the theoretical mass of the diastereomer salt:

$$Y_R = \frac{m_R - m_p}{m_R}$$

The enantiomer excess (ee) was calculated as:

$$ee = \frac{|A_D - A_L|}{A_D + A_L}$$

The ee was determined by chiral GC. $A$ denotes peak areas on the chromatograms while $D$ and $L$ signify the two enantiomers. Based on our experiences, this determination method has an error of 3 % ee.

From the ee and the yield values, the dimensionless $S$ (selectivity) parameter can be calculated to describe the efficiency of the entire process [9]:

---

1) List of symbols at the end of the paper.
\[ S_R = Y_R \times \text{ee}_R \] (6)

where the index R refers to the raffinate. Since both the yield and the ee of the raffinate vary from 0 to 1, selectivity also falls between 0 and 1 and is equal to 1 if the separation is complete.

3 Results and Discussion

3.1 Resolution of Racemic CPA

The in situ resolution of CPA could not be realized using PhEA: no enantiomer selectivity was observed in the CO\(_2\) phase; however, the raffinate yields were high varying between 82 % and 100 % with ee < 10 %. PhEA easily reacts with CO\(_2\) by forming a carbamate-type salt [10], however, XRD measurements showed that the formed compound is not the PhEA-CO\(_2\) carbamate. Thus, the obtained high yield and the low ee values indicate the nonselective formation of the diastereomer salt, i.e., PhEA reacts with both enantiomers. In contrast, extracting the unreacted enantiomer from the CPA-PhEA mixture formed in vacuo provided a diastereomer salt with an ee of 25 % independent of reaction time at 200 bar and 45 °C.

With BAB, the chiral resolution can be carried out via both processes, thus the reaction was studied in situ in the range of 150–215 bar and 35–55 °C using BAB. The highest selectivity, 0.40, was obtained at 200 bar and 35 °C. The pressure dependence of the system measured at 45 °C is illustrated in Fig. 3.

At the lowest pressure of 150 bar, no enantiomer selectivity was achieved. The ee of the raffinate slightly increases between 170 and 215 bar. The yield shows a slightly decreasing tendency with increasing pressure, presumably due to the higher density of CO\(_2\): more diastereomer salt can be extracted, however, salt remaining in the raffinate has a slightly higher ee. The best separation was achieved at 215 bar. Further investigations of the effect of temperature demonstrated that if the temperature is increased to 60 °C, the raffinate could only be recovered in liquid form with zero ee. The temperature dependence of the system at 200 bar is indicated in Tab. 1.

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<th>T [°C]</th>
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<th>Y(_R) [%]</th>
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<tr>
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XRD measurements proved that the diastereomer salt formed with this technique had a crystal structure different from diastereomer salts obtained with the in vacuo method. Presumably, in the technique a quick kinetic control dominates which causes a different structure from the in situ method, the structures of which matched literature data, whereas thermodynamic control could govern the reaction during the long-term salt formation. The differences are displayed in Fig. 4.

3.2 Resolution of Racemic CCA

Experiments were performed at 80, 100, and 125 bar pressure and at 33 °C, 40 °C, and 47 °C temperature. PhEA provided a racemic product in preliminary experiments. Since no significant differences between the results of the in situ and in vacuo methods were observed during preliminary experimental work performed at 100 bar and 33 °C, results presented here were obtained in situ using BAB. In each experiment, samples which were taken from the CO\(_2\) phase during the reaction showed a constant ee value throughout the reaction time. This behavior indicates that a quick dynamic equilibrium evolved between the CO\(_2\) and solid phases which remained constant thereafter. Fig. 5 presents the average sample ee values in the CO\(_2\) phase versus temperature and pressure when using BAB as the resolving agent.

Generally, samples which were collected by washing out the entire reactor volume after the reaction have lower ee compared...
to values obtained by sampling the reaction mixture. This phenomenon becomes more prominent at higher pressures according to Fig. 6. At 40 °C and 125 bar the experiment produced an almost racemic extract composition after washing (ee ~ 10%), which indicates that the diastereomer salt loses its stability and decomposes to the enantiomer and to the resolving agent, thus decreasing the extract ee while increasing its yield.

Raffinate yield ($Y_R$) decreases with higher CO2 density. The highest ee and the highest selectivity of 0.46 were obtained at 100 bar and 40 °C with $Y_R = 0.61$.

Optical view cell measurements demonstrated that the solubility of the enantiomers dramatically increases around 0.62 g mL$^{-1}$ CO2 density. Below this limit, enantiomer solubility determines the sample ee values: the greater the solubility, the better the separation during extraction, thus the ee increases. Above this density of 0.62 g mL$^{-1}$, CCA is completely dissolved and no further increase in separation efficiency occurs. High densities here result in a decreased stability of the diastereomer salt though, thus the raffinate ee is decreasing; see Fig. 7.

### 4 Conclusions

The organic solvent-free optical resolution of two racemic cyclopropanecarboxylic acids was realized by applying the modified Pope-Peachy method and using scCO2 as reaction medium. PhEA did not prove to be a good resolving agent, while BAB was able to resolve both acids, albeit in different pressure ranges: the optimal circumstances for resolving CCA were at 40 °C and 100 bar, yielding a selectivity value of 0.46. The resolution of CPA at 200 bar and at 35 °C resulted in a selectivity value of 0.40 in the raffinate. In all cases, a complete conversion was achieved in less than 1 h. XRD analysis showed that the CPA-BAB diastereomer salt formed with the in vacuo technique had a crystal structure different from the diastereomer salts obtained in situ, which is the structure of which matched literature data, probably due to the role of kinetic and thermodynamic control.

### Acknowledgment

This work was supported by a grant from the Hungarian Scientific Research Fund (grant no. K 108979) as well as by Gideon Richter Plc. via the Gideon Richter PhD Scholarship. J. M. would like to thank the ERASMUS program for financial support, the work of E. S. was supported by the Bolyai Janos Research Fund.

The authors have declared no conflict of interest.

### Symbols used

- $A$ [mV s] area of the peak obtained by GC
- ee [–] enantiomeric excess
- $m$ [g] mass
- $mr$ [–] molar ratio
- $n$ [mol] molar amount
Indices

<table>
<thead>
<tr>
<th>Index</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>initial</td>
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<tr>
<td>E</td>
<td>extract</td>
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<tr>
<td>p</td>
<td>Perfil (inert porous supporting material)</td>
</tr>
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<td>R</td>
<td>raffinate</td>
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<td>rac</td>
<td>racemic acid</td>
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<tr>
<td>res</td>
<td>resolving agent</td>
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Abbreviations

- BAB: (S)-2-benzylamino-1-butanol
- CCA: 3-(2,2-dimethylvinyl)-2,2-dimethylcyclopropane-carboxylic acid
- CPA: 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid
- DDT: 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane
- SFE: Supercritical fluid extraction
- PhEA: (R)-1-phenylethaneamine
- XRD: X-ray powder diffraction

References

APPENDIX

A List of Symbols

A peak area
c volumetric concentration
F resolution efficiency, relative to total amount (Eq. 2.17)
F̂ resolution efficiency, ideal CSTR model (Eq. 2.18)
k number of chiral centers
m mass
ṁ mass flow rate
m̃ theoretical mass (Eqs. 2.13 and 2.14)
M molar mass
n molar quantity
p pressure
R CO₂:solvent mass ratio
R_m CO₂:solvent molar ratio
ρ density
Q generic quantity (see Eq. 1.1)
S selectivity
t time
T temperature
2Θ scattering angle
V volume
x saturation concentration
Y yield, relative to total amount (Eq. 2.6)
Ŷ yield, ideal resolution model (Eq. 2.7)
Ŷ yield, ideal CSTR model (Eq. 2.15)

Indices

(R) major component is (R)-IBU
(S) major component is (S)-IBU
(+) major component is (+)-cPA
(−) major component is (−)-cPA

0 initial
c critical
d dissolution point
e extract
i fraction, running index
maj major component
min minor component
o cloud point
r raffinate
rac racemate
res resolving agent

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<td>BAB</td>
<td>2-(N-benzylamino)butan-1-ol</td>
</tr>
<tr>
<td>(S)-BAB</td>
<td>(S)-(+)2-(N-benzylamino)butan-1-ol</td>
</tr>
<tr>
<td>cPA</td>
<td>cis-permethric acid</td>
</tr>
<tr>
<td>(+)-cPA</td>
<td>(1R,3R)-(+)cis-permethric acid</td>
</tr>
<tr>
<td>(−)-cPA</td>
<td>(1S,3S)-(−)cis-permethric acid</td>
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<td>IBU</td>
<td>ibuprofen</td>
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<tr>
<td>(R)-IBU</td>
<td>(R)-(−)-ibuprofen</td>
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<tr>
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<td>(PhEA){sub}CO_{sub}2</td>
<td>1-phenylethananminium (1-phenylethyl)carbamate</td>
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ee enantiomeric excess
de diastereomeric excess
ee̅ averaged enantiomeric excess
GAS gas antisolvent
GC gas chromatography
mr molar ratio
SAS supercritical antisolvent
SEM scanning electron microscopy
XRD powder X-ray diffraction
**Figure A1:** Originals of SEM images presented in Fig. 26 (Section 3.1.2, p. 62).
# Supplementary Data

**Table A1:** Supplementary data for Figure 19. *In situ* resolution of IBU with (R)-PhEA. Effects of pressure ($p$) and reaction time ($t$) at 40 °C.

<table>
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Table A2: Supplementary data for Figure 20. *In situ* resolution of IBU with \((R)\)-PhEA. Effects of temperature \((T)\) at 200 bar. \(t\) denotes time of sampling.

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<td>IBUE-67</td>
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<td>1.0</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td></td>
<td>73.0</td>
<td>0.70</td>
</tr>
<tr>
<td>IBUE-68</td>
<td>50</td>
<td>99.0</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td>123.0</td>
<td>0.78</td>
</tr>
<tr>
<td>IBUE-78</td>
<td>40</td>
<td>1.5</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.5</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25.0</td>
<td>0.20</td>
</tr>
<tr>
<td>IBUE-87</td>
<td>40</td>
<td>93.5</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>94.0</td>
<td>0.39</td>
</tr>
<tr>
<td>IBUE-86</td>
<td>40</td>
<td>1.0</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.0</td>
<td>0.21</td>
</tr>
</tbody>
</table>

Table A3: Supplementary data for Figures 21 and 22. GAS resolution of IBU with \((R)\)-PhEA. Effects of pressure \((p)\) at 45 °C, using 2 ml of methanol as solvent.

<table>
<thead>
<tr>
<th>code</th>
<th>(p [\text{bar}])</th>
<th>(Y [-])</th>
<th>(\text{ee}_{(S)} [-])</th>
<th>(Y [-])</th>
<th>(\text{ee}_{(R)} [-])</th>
<th>(F [-])</th>
</tr>
</thead>
<tbody>
<tr>
<td>SASS-02</td>
<td>100</td>
<td>0.29</td>
<td>0.59</td>
<td>0.36</td>
<td>0.75</td>
<td>0.44</td>
</tr>
<tr>
<td>SASS-14</td>
<td>120</td>
<td>0.30</td>
<td>0.44</td>
<td>0.42</td>
<td>0.74</td>
<td>0.44</td>
</tr>
<tr>
<td>SASS-15</td>
<td>130</td>
<td>0.28</td>
<td>0.33</td>
<td>0.35</td>
<td>0.76</td>
<td>0.36</td>
</tr>
<tr>
<td>SASS-06</td>
<td>140</td>
<td>0.35</td>
<td>0.33</td>
<td>0.33</td>
<td>0.75</td>
<td>0.36</td>
</tr>
<tr>
<td>SASS-12</td>
<td>150</td>
<td>0.35</td>
<td>0.34</td>
<td>0.33</td>
<td>0.73</td>
<td>0.36</td>
</tr>
<tr>
<td>SASS-08</td>
<td>160</td>
<td>0.28</td>
<td>0.26</td>
<td>0.29</td>
<td>0.73</td>
<td>0.28</td>
</tr>
<tr>
<td>SASS-09</td>
<td>180</td>
<td>0.33</td>
<td>0.24</td>
<td>0.30</td>
<td>0.77</td>
<td>0.31</td>
</tr>
<tr>
<td>SASS-10</td>
<td>190</td>
<td>0.27</td>
<td>0.16</td>
<td>0.20</td>
<td>0.79</td>
<td>0.20</td>
</tr>
<tr>
<td>SASS-13</td>
<td>210</td>
<td>0.36</td>
<td>0.23</td>
<td>0.20</td>
<td>0.77</td>
<td>0.24</td>
</tr>
</tbody>
</table>
Table A4: Supplementary data for Figures 23, 24, 25 and 49. GAS resolution of IBU with (R)-PhEA. Effects of CO₂–solvent ratio (R or \( R_m \)) at 150 bar and 45 °C, using 2 ml solvent.

<table>
<thead>
<tr>
<th>code</th>
<th>solvent</th>
<th>( R ) [g/g]</th>
<th>( R_m ) [mol/mol]</th>
<th>( Y ) [-]</th>
<th>ee(_{(R)}) [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAS-80</td>
<td>MeOH</td>
<td>29.5</td>
<td>21.5</td>
<td>0.43</td>
<td>0.78</td>
</tr>
<tr>
<td>SAS-87</td>
<td>MeOH</td>
<td>19.3</td>
<td>14.1</td>
<td>0.36</td>
<td>0.66</td>
</tr>
<tr>
<td>SAS-78</td>
<td>MeOH</td>
<td>13.8</td>
<td>10.0</td>
<td>0.21</td>
<td>0.85</td>
</tr>
<tr>
<td>SAS-89</td>
<td>MeOH</td>
<td>14.3</td>
<td>10.4</td>
<td>0.28</td>
<td>0.72</td>
</tr>
<tr>
<td>SAS-77</td>
<td>MeOH</td>
<td>11.0</td>
<td>8.0</td>
<td>0.11</td>
<td>0.73</td>
</tr>
<tr>
<td>SAS-79</td>
<td>MeOH</td>
<td>8.7</td>
<td>6.3</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>SAS-84</td>
<td>EtOH</td>
<td>14.4</td>
<td>15.1</td>
<td>0.41</td>
<td>0.84</td>
</tr>
<tr>
<td>SAS-82</td>
<td>EtOH</td>
<td>11.2</td>
<td>11.7</td>
<td>0.34</td>
<td>0.91</td>
</tr>
<tr>
<td>SAS-83</td>
<td>EtOH</td>
<td>9.5</td>
<td>9.9</td>
<td>0.30</td>
<td>0.87</td>
</tr>
<tr>
<td>SAS-86</td>
<td>EtOH</td>
<td>7.9</td>
<td>8.3</td>
<td>0.20</td>
<td>0.82</td>
</tr>
<tr>
<td>SAS-85</td>
<td>EtOH</td>
<td>7.0</td>
<td>7.3</td>
<td>0.11</td>
<td>0.80</td>
</tr>
<tr>
<td>SAS-88</td>
<td>MeOH+EtOH</td>
<td>14.2</td>
<td>12.6</td>
<td>0.34</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Table A5: Supplementary data for Figures 28, 29 and 30. GAS resolution of IBU with (R)-PhEA. Effects of molar ratio (mr) at 130 bar and 45 °C.

<table>
<thead>
<tr>
<th>code</th>
<th>mr</th>
<th>( Y ) [-]</th>
<th>ee(_{(S)}) [-]</th>
<th>( Y ) [-]</th>
<th>ee(_{(R)}) [-]</th>
<th>( F )</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBU-M-17</td>
<td>0.313</td>
<td>0.87</td>
<td>0.14</td>
<td>0.18</td>
<td>0.76</td>
<td>0.26</td>
</tr>
<tr>
<td>IBU-M-16</td>
<td>0.416</td>
<td>0.67</td>
<td>0.24</td>
<td>0.26</td>
<td>0.77</td>
<td>0.36</td>
</tr>
<tr>
<td>SASS-15*</td>
<td>0.501</td>
<td>0.28†</td>
<td>0.33</td>
<td>0.35</td>
<td>0.76</td>
<td>0.36</td>
</tr>
<tr>
<td>IBU-M-2</td>
<td>0.503</td>
<td>0.54</td>
<td>0.34</td>
<td>0.36</td>
<td>0.72</td>
<td>0.45</td>
</tr>
<tr>
<td>IBU-M-3</td>
<td>0.522</td>
<td>0.48</td>
<td>0.28</td>
<td>0.34</td>
<td>0.74</td>
<td>0.39</td>
</tr>
<tr>
<td>IBU-M-21</td>
<td>0.615</td>
<td>0.25</td>
<td>0.30</td>
<td>0.36</td>
<td>0.77</td>
<td>0.35</td>
</tr>
<tr>
<td>IBU-M-11</td>
<td>0.761</td>
<td>0.18</td>
<td>0.51</td>
<td>0.54</td>
<td>0.29</td>
<td>0.25</td>
</tr>
<tr>
<td>IBU-M-12</td>
<td>1.010</td>
<td>0.17</td>
<td>0.54</td>
<td>0.72</td>
<td>0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>IBU-M-13</td>
<td>1.261</td>
<td>0.18</td>
<td>0.52</td>
<td>0.61</td>
<td>0.21</td>
<td>0.22</td>
</tr>
</tbody>
</table>

* Included from Table A3.
† Outlier due to possible inconsistency in experimental technique, for calculation of \( F \) this value was replaced with the average of the respective values from IBU-M-2 and IBU-M-3.
**Table A6:** Supplementary data for Figure 31. GAS resolution of IBU with (S)-PhEA. Effects of initial ee (ee\textsubscript{0}) at 130 bar, 45 °C and mr = 0.5.

<table>
<thead>
<tr>
<th>code</th>
<th>method</th>
<th>mr</th>
<th>ee\textsubscript{0} [-]</th>
<th>extract ee [-]</th>
<th>raffinate ee [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBU-M-3*\†</td>
<td></td>
<td></td>
<td>0.000\‡</td>
<td>0.28 (S)</td>
<td>0.74 (R)</td>
</tr>
<tr>
<td>IBU-M-2*\†</td>
<td></td>
<td></td>
<td>0.000\‡</td>
<td>0.34 (S)</td>
<td>0.72 (R)</td>
</tr>
<tr>
<td>IBU-M-7\†</td>
<td></td>
<td></td>
<td>0.000\‡</td>
<td>0.29 (S)</td>
<td>0.61 (R)</td>
</tr>
<tr>
<td>IBU-M-8\†</td>
<td></td>
<td></td>
<td>0.000\‡</td>
<td>0.36 (S)</td>
<td>0.48 (R)</td>
</tr>
<tr>
<td>IBU-M-22</td>
<td></td>
<td></td>
<td>0.186 (S)</td>
<td>0.11 (R)</td>
<td>0.76 (S)</td>
</tr>
<tr>
<td>IBU-M-23</td>
<td></td>
<td></td>
<td>0.422 (S)</td>
<td>0.16 (S)</td>
<td>0.86 (S)</td>
</tr>
<tr>
<td>IBU-M-18</td>
<td></td>
<td></td>
<td>0.776 (S)</td>
<td>0.76 (S)</td>
<td>0.93 (S)</td>
</tr>
</tbody>
</table>

\*Included from Table A5.
\†Experiments using (R)-PhEA, these were averaged and used as an estimate according to the Marckwald principle.
\‡Estimated for racemate, ee is below GC detection limits.

**Table A7:** Supplementary data for Figure 32. SAS resolution of IBU with (R)-PhEA. Two-step purification experiment at 130 bar, 45 °C. Value in parentheses estimated, assuming SAS raffinates contain equimolar diastereomers.

<table>
<thead>
<tr>
<th>code</th>
<th>method</th>
<th>mr</th>
<th>ee\textsubscript{0} [-]</th>
<th>extract Y [-]</th>
<th>ee [-]</th>
<th>raffinate Y [-]</th>
<th>ee [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBU-M-26</td>
<td>SAS</td>
<td>1.005</td>
<td>0.000*</td>
<td>0.24</td>
<td>0.32 (S)</td>
<td>0.23</td>
<td>0.51 (R)</td>
</tr>
<tr>
<td>IBU-M-27</td>
<td>GAS</td>
<td>1.000\†</td>
<td>0.51 (R)</td>
<td>0.25</td>
<td>0.07 (R)</td>
<td>0.47</td>
<td>0.90 (R)</td>
</tr>
</tbody>
</table>

\*Estimated for racemate, ee is below GC detection limits.
\†Estimated, raffinate assumed to contain equimolar diastereomers.

**Table A8:** Supplementary data for Figure 33. In situ resolution of cPA with (S)-BAB. Effect of reaction time at 200 bar, 45 °C. \( t \) denotes time of sampling.

<table>
<thead>
<tr>
<th>code</th>
<th>( t ) [h]</th>
<th>ee\textsubscript{(+)} [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPSD-7b</td>
<td>1</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>44</td>
<td>0.29</td>
</tr>
<tr>
<td>CPSD-14b</td>
<td>1</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>0.23</td>
</tr>
</tbody>
</table>
Table A9: Supplementary data for Figure 34. *In situ* resolution of cPA with (S)-BAB. Effect of pressure ($p$) at 45 °C.

<table>
<thead>
<tr>
<th>code</th>
<th>$p$ [bar]</th>
<th>$\hat{Y}$ [-]</th>
<th>ee$_{(-)}$ [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPSD-18b</td>
<td>215</td>
<td>0.59</td>
<td>0.78</td>
</tr>
<tr>
<td>CPSD-04b</td>
<td>200</td>
<td>0.55</td>
<td>0.68</td>
</tr>
<tr>
<td>CPSD-19b</td>
<td>190</td>
<td>0.63</td>
<td>0.70</td>
</tr>
<tr>
<td>CPSD-20b</td>
<td>180</td>
<td>0.61</td>
<td>0.69</td>
</tr>
<tr>
<td>CPSD-21b</td>
<td>170</td>
<td>0.61</td>
<td>0.64</td>
</tr>
<tr>
<td>CPSD-22b</td>
<td>160</td>
<td>0.63</td>
<td>0.41</td>
</tr>
<tr>
<td>CPSD-05b</td>
<td>150</td>
<td>0.59</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Figure A2: *In situ* resolution of cPA with (S)-BAB. Diffractogram of the raffinate obtained at 200 bar and 45 °C, compared against the diffractograms of an enantiopure standard prepared by vacuum evaporation (labelled (−)-cPA–(S)-BAB).
Table A10: Supplementary data for Figures 36 and 37. GAS resolution of cPA with (R)-PhEA. Effect of pressure ($p$) at 45 °C.

<table>
<thead>
<tr>
<th>code</th>
<th>$p$ [bar]</th>
<th>$Y$ [-]</th>
<th>ee_{(-)} [-]</th>
<th>$\delta$ [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAS-12</td>
<td>100</td>
<td>0.61</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>SAS-27</td>
<td>110</td>
<td>0.60</td>
<td>0.05</td>
<td>0.03</td>
</tr>
<tr>
<td>SAS-12</td>
<td>120</td>
<td>0.46</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>SAS-25</td>
<td>130</td>
<td>0.39</td>
<td>0.91</td>
<td>0.36</td>
</tr>
<tr>
<td>SAS-30</td>
<td>150</td>
<td>0.32</td>
<td>0.94</td>
<td>0.30</td>
</tr>
<tr>
<td>SAS-23</td>
<td>160</td>
<td>0.32</td>
<td>0.83</td>
<td>0.26</td>
</tr>
<tr>
<td>SAS-22</td>
<td>170</td>
<td>0.28</td>
<td>0.90</td>
<td>0.25</td>
</tr>
<tr>
<td>SAS-20</td>
<td>180</td>
<td>0.03</td>
<td>0.26</td>
<td>0.01</td>
</tr>
<tr>
<td>SAS-19</td>
<td>190</td>
<td>0.06</td>
<td>0.26</td>
<td>0.02</td>
</tr>
<tr>
<td>SAS-18</td>
<td>200</td>
<td>0.08</td>
<td>0.34</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Table A11: Supplementary data for Figures 42, 43 and 49. SAS resolution of cPA with (R)-PhEA. Effect of the CO$_2$:solvent ratio ($R$) at 150 bar, 45 °C.

<table>
<thead>
<tr>
<th>code</th>
<th>$R$ [g/g]</th>
<th>$Y$ [-]</th>
<th>ee_{(-)} [-]</th>
</tr>
</thead>
<tbody>
<tr>
<td>SASG-23</td>
<td>11.4</td>
<td>0.04</td>
<td>0.94</td>
</tr>
<tr>
<td>SASG-26</td>
<td>12.0</td>
<td>0.07</td>
<td>0.90</td>
</tr>
<tr>
<td>SASG-27</td>
<td>12.0</td>
<td>0.09</td>
<td>0.89</td>
</tr>
<tr>
<td>SASG-20</td>
<td>13.0</td>
<td>0.11</td>
<td>0.95</td>
</tr>
<tr>
<td>SASG-21</td>
<td>13.0</td>
<td>0.15</td>
<td>0.93</td>
</tr>
<tr>
<td>SASG-22</td>
<td>14.7</td>
<td>0.25</td>
<td>0.87</td>
</tr>
</tbody>
</table>